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FINAL TECHNICAL REPORT
NGR-33-010-179

CENTRAL NEURAL MECHANISMS
GOVERNING POSTURAL
CARDIOVASCULAR MECHANISMS

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August, 1976

(NASA-CP-148603) CENTRAL NEURAL MECHANISMS
GOVERNING POSTURAL CARDIOVASCULAR MECHANISMS
Final Technical Report, 15 Jun. 1972 - 14
Jun. 1976 (Cornell Univ., New York.) 293 p
HC \$9.25

N76-29898

Unclas
CSCL 06P G3/52 48888



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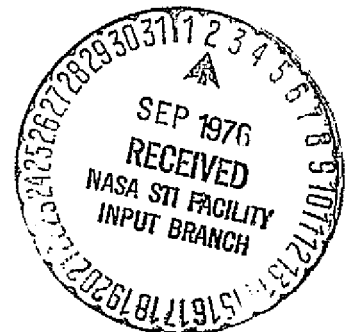
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Project Period

June 15, 1972 - June 14, 1976

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FINAL TECHNICAL REPORT FOR NASA

I. INTRODUCTION AND RESEARCH HIGHLIGHTS

Summarized below are the results of studies in the Laboratory of Neurobiology supported by Grant NGR-33-010-179 from NASA. While the initial purpose of the proposal was to study the role of the vestibular apparatus and cerebellum in orthostatic reflex control, our investigation led us further into elaborating on mechanisms within the brain which govern circulation reflexes and the consequences of disturbances in their function.

Research Highlights

The research activities have been divided into three major categories, A - C, which are presented in detail below. This section will only highlight their significance.

Group A: Studies of Cerebellar and Vestibular Mechanisms Governing the Circulation and Orthostatic Reflexes.

This group consists of a number of studies investigating the interaction between areas of the brain governing positional and cardiovascular reflexes. The major findings have been: (a) the discovery that electrical stimulation in a restricted portion of a subnucleus of the cerebellum, the fastigial nucleus, can elicit powerful circulatory changes simulating orthostatic responses; (b) that lesions of the fastigial nucleus will impair orthostatic responses; (c) that vestibular inputs, acting in concert with baroreceptors and the cerebellum, also contribute to orthostasis; and (d) the discovery that stimulation of this area of the fastigial nucleus will produce a wide range of emotional behaviors, as well as influence the circulation, points to a new function of the cerebellum, a brain region heretofore believed to be involved primarily in the control of posture. In view of the intimate interaction between autonomic and postural mechanisms in weightlessness, this knowledge may be of importance in designing strategies for pharmacological control of circulatory decompensation following prolonged weightlessness.

Group B: Studies of Central Baroreceptor Integration in the Nucleus Tractus Solitarii (NTS) and Anterior Hypothalamus (AH).

The NTS and AH are two brain regions of importance in baroreceptor reflexes, and hence, are of relevance to orthostatic reflex control. We discovered that: (a) lesions of the NTS in rats not only abolish baroreceptor reflexes, but result in the development of fulminating neurogenic

hypertension and pulmonary edema. Further investigations in rat demonstrated that the syndrome is associated with release of adrenal catecholamines and depends on central neural pathways releasing norepinephrine. (b) Recent study in cats have demonstrated that this species, unlike rats, will survive the acute phase of hypertension and enter a chronic stage of chronic labile neurogenic hypertension. (c) Lesions of the anterior hypothalamus in rat will also produce fulminating hypertension and pulmonary edema resulting from release of adrenal catecholamines rather than increased vasomotor activity.

These studies also serve to cast light on the function of brain regions subserving orthostasis and baroreceptor mechanisms. From the point of space flight, they are of importance in highlighting regions which can be studied pharmacologically to develop arterial modes of control of circulatory reflexes. They are also of importance in a broader sense biomedically in demonstrating, for the first time, that lesions of the brain producing imbalance in pressor and depressor centers, can lead to chronic hypertension.

Group C: Studies on Brainstem Mechanisms Controlling Circulatory Reflexes Other Than Arterial Baroreceptor Responses.

The major findings were the discoveries of the regions of the brain integrating the Cushing response (the elevation of arterial pressure resulting from increased intracranial pressure), and the cerebral ischaemic reflex (the pressor response to rendering the brain ischaemic). The region for the two responses appears localized to the upper medulla. It also appears to represent the tonic vasomotor center of brain.

Another finding was the discovery of another and previously unrecognized major depressor area of the brain within the spinal trigeminal complex. The response elicited from this depressor system has been termed the "trigeminal depressor response (TDR)". The TDR is a reflex from receptors of the trigeminal nerve and also from receptors, as yet unknown, innervated by branches of the glossopharyngeal and vagus nerves.

An understanding of other systems of brain reflexes governing the arterial pressure is obviously of importance in designing other strategies for examining the reflex control of the circulation under conditions of weightlessness.

Group D: Other Related Studies and Reviews.

This group consists of a technical report and several review articles incorporating much material submitted as primary research projects.

II. SUMMARY OF RESEARCH

GROUP A: STUDIES OF CEREBELLAR AND VESTIBULAR MECHANISMS GOVERNING THE CIRCULATION AND ORTHOSTATIC REFLEXES.

1. Doba, N. and Reis, D.J.: Changes in regional blood flow and cardiodynamics evoked by electrical stimulation of the fastigial nucleus in the cat and their similarity to orthostatic reflexes. *J. Physiol.* 227: 729-747, 1972.

Changes in regional blood flow and cardiodynamics were measured in anesthetized paralyzed cats during electrical stimulation of the rostral fastigial nucleus. Fastigial stimulation results in a graded, highly reproducible and stereotyped cardiovascular response characterized by (a) increased systolic, diastolic and mean arterial pressures without changes in central venous or occluded vein pressure, (b) decreased blood flow and increased vascular resistance in the axillary, renal, femoral and mesenteric arteries, increased flow without any change in vascular resistance in the common carotid artery, and increase in total peripheral resistance, (c) a small increase in heart rate and myocardial contractile force, decrease in calculated stroke volume, and no change in the cardiac output. Changes in regional arterial flow were abolished by transection of sympathetic nerves or blockade of α -adrenergic receptors by systemic administration of phentolamine. Changes in heart rate and myocardial contractility were abolished by stellate ganglionectomy or blockade of β -adrenergic receptors by propranolol. No changes in pupillary diameter or retraction of the nictitating membrane were seen during fastigial stimulation with stimuli producing substantial changes in blood pressure. The fastigial pressor response represents a highly reproducible, stereotyped, graded, and differentiated pattern of activation of sympathetic preganglionic neurons. The pattern of cardiovascular effects of fastigial stimulation simulates the compensatory (orthostatic) reflex response to maintenance of an upright posture. Fastigial stimulation appears to excite the neural network subserving orthostatic reflexes.

2. Doba, N. and Reis, D.J.: The role of the cerebellum and vestibular apparatus in regulation of orthostatic reflexes in the cat. *Circ. Res.* 34: 9-18, 1974.

The contribution of the fastigial nucleus and the vestibular nerves (VIIIth cranial nerves) to the orthostatic reflexes in anesthetized, paralyzed cats was studied. Bilateral lesions of the rostral fastigial nucleus resulted in impairment of the reflex changes in blood pressure, femoral arterial flow and resistance evoked by head-up

tilting to 30° or 60°. The deficit consisted of an increase in the magnitude of the initial fall in blood pressure during tilting. The effects on blood pressure were paralleled by decreased vasoconstriction in the femoral artery. Extracranial lesions of the vestibular nerves produced comparable deficits which were not enhanced by subsequent lesions of the fastigial nucleus. Denervation of the baroreceptors impaired the reflexes, and subsequent lesion of the fastigial nucleus increased this deficit. The pressor response evoked by electrical stimulation of the rostral fastigial nucleus also reversed the deficit in orthostasis produced by hemorrhage. Small doses of sodium pentobarbital which did not alter the resting blood pressure or the pressor response to carotid occlusion impaired the responses to stimulation of the fastigial nucleus and tilting. Therefore, the rostral fastigial nucleus, which might be triggered by the vestibular apparatus, appears to participate in concert with the baroreceptors in the initiation and possibly the maintenance of the orthostatic reflexes.

3. Reis, D.J., Doba, N. and Nathan, M.A.: Predatory attack, grooming and consummatory behavior evoked by electrical stimulation of cat cerebellar nuclei. *Science* 182: 845-847, 1973.

In this study, we sought to determine the behavioral consequences of electrical stimulation of sites of the fastigial nucleus from which the cardiovascular changes simulating the orthostatic reflexes were elicited. The relevance of this study to the aims of NASA are that, if theoretically one wished to activate fastigial mechanisms in attempting to compensate for orthostasis in alert, unanesthetized subjects, the associated concomitants of such stimulation should be known.

Electrical stimulation ventro-medially in the rostral fastigial nucleus (FN) of anesthetized cat evokes a profound rise of blood pressure and tachycardia, the fastigial pressor response (FPR). To examine if the FPR is associated with behavioral changes, stimulating electrodes were chronically implanted in FN of cat along with an indwelling cannula in the carotid artery. Electrical stimulation restricted to FN (50 cps for 30-60 sec) in awake cat at intensities just above threshold for hypertension most commonly elicited alerting and vocalization. At slightly higher intensities, the cats exhibited a range of stimulus-locked behaviors including intense grooming, predatory (biting) attack on rats, eating or drinking. The behavioral responses were stereotyped, coordinated and characteristic for each animal. No abnormalities of posture or movement were noted. Lesions at electrode tips abolished the behavior and pressor responses without producing motor deficits or impairment of defensive responses to attack or pain. We conclude that the rostral fastigial nucleus can modulate behaviors such as aggression,

feeding and drinking heretofore considered as preponderantly organized in upper brainstem and limbic system.

4. Sheridan, G. and Reis, D.J.: Effects of cerebellar ablation on regional distribution of cardiac output in cat. Brain Res. 45: 260-265, 1972.

It has long been known that the cerebellum phasically influences the circulation. Electrical stimulation of the cerebellar cortex or deep nuclei has produced transient changes in blood pressure, heart rate, the distribution of organ blood flow, sensitivity of baroreceptors and other cardiovascular reflexes. In the present study we have investigated whether the cerebellum may exert a tonic influence on circulation by investigating the effects of total cerebellar ablation in the cat, both acutely and chronically, on the distribution of nutrient (non-shunt) organ blood flow.

The studies were performed on cat. Under anesthesia the cerebellum was removed completely. The fractional distribution of blood flow was measured in 5 cats three days after cerebellectomy (sub-acute group) and in 4 cats twenty-eight days after surgery (chronic group). Blood flow distribution in skeletal muscle and viscera of unanesthetized cat was measured by our modification of the isotope dilution method of Sapirstein using ^{86}Rb as an indicator.

The results demonstrated that the only significant change in regional blood flow distribution of limbs was a decrease in the distribution of blood flow to red skeletal muscles 3 days after cerebellectomy. As a result of the reduced fractional flow to red muscle the 3:1 ration of red to white blood flow characteristic of quiet waking approached unity. The decrease of fractional flow to red muscles was transient, since by 28 days it had returned to normal. The study therefore demonstrates that despite the profound abnormalities in motor activity resulting from cerebellectomy, changes in the regional distribution of blood flow are small and only transiently alter the distribution of blood flow to red skeletal muscle. The finding that the cerebellum may tonically regulate the distribution of blood flow to red skeletal muscle is not surprising in view of the important role of the cerebellum in governing posture and the selective participation of red skeletal muscle in this activity.

5. Kumada, M. and Reis, D.J.: Cardiovascular effects elicited by punctate electrical stimulation within the vestibular complex (in preparation).

We have sought to identify regions within the vestibular nuclear complex which might relay the orthostatic responses previously discovered to be initiated from vestibular apparatus in cat. Studies were undertaken in 10 cats and 20 rabbits anesthetized with alpha-chlorase (50 mg/kg) after

induction with ether. Some rabbits were anesthetized by urethane (1 mg/kg i.v.). The brainstem was stimulated with Teflon-coated electrodes with a constant current source. Over 180 histologically verified tracks were placed and 1,728 sites were stimulated while arterial pressure and heart rate were continuously displayed on channels of a polygraph. Both pressor and depressor responses could be elicited by electrical stimulation of areas within and immediately surrounding the vestibular nuclear complex. A pressor response was elicited from the ventral border of the medial vestibular nucleus and adjacent dorsal reticular formation. The response consisted of a rise in both systolic and diastolic arterial pressures to as much as 100 mmHg. The threshold for the response was less than 20 μ A and the optimal stimulus frequency was over 50 Hz. The response was associated with a bradycardia which, following denervation of arterial baroreceptors, reverted to a tachycardia. The pressor response appeared similar to the so-called Cushing response on the basis of a comparable pattern of circulatory changes and a substantial overlap of the CNS sites from which the two responses were elicited.

A depressor response was elicited from stimulation of the lateral and descending vestibular nuclei. It consisted of a fall in both systolic and diastolic pressures by 25 mmHg or less associated with a bradycardia. The threshold for the response within the vestibular nucleus was usually greater than 100 μ A with an optimal frequency of 5 to 20 Hz. It is probable that the site from which this depressor response is elicited lies outside the vestibular nuclear complex within the spinal tract of the trigeminal nerve (since when an electrode was lowered through the vestibular complex into the trigeminal complex, the threshold was substantially lowered). Stimulation of the superior vestibular nucleus failed to elicit any cardiovascular changes. We conclude that electrical stimulation within the vestibular nucleus complex produces few cardiovascular changes which can conclusively be attributed to that nucleus. The pressor response appears to be elicited primarily from structures in the dorsal tegmental pontine reticular formation while the depressor response is probably mediated by spread of current to underlying spinal trigeminal tract. Failure to elicit cardiovascular effects from electrical stimulation within the vestibular complex might be due to either dispersion of fibers having the cardiovascular influence throughout the complex itself, or that fibers from the VIIIth nerve involved in cardiovascular control pass in small bundles directly to the fastigial nucleus of cerebellum and/or regions of the reticular formation.

GROUP B: STUDIES OF CENTRAL BARORECEPTOR INTEGRATION
IN THE NUCLEUS TRACTUS SOLITARI (NTS) AND
ANTERIOR HYPOTHALAMUS WITH REFERENCE TO
CHRONIC EFFECTS OF LOCAL LESIONS.

1. Doba, N. and Reis, D.J.: Acute fulminating neuro-
genic hypertension produced by brainstem lesions in rat.
Circ. Res. 32: 584-593, 1973.

Bilateral electrolytic lesions of the nucleus tractus solitarius in the rat at the level of the obex abolished baroreceptor reflexes and resulted in an immediate, marked elevation in systemic blood pressure without a change in heart rate. In unanesthetized rats, the hypertension was associated with a marked increase in total peripheral resistance, a reduction in blood flow in the abdominal aorta, and an increase in central venous pressure. The cardiac output was reduced to 62% of control as a consequence of reduced stroke volume, which was reflected, in turn, by increased end-diastolic pressure. The hypertension was abolished and the end-diastolic pressure lowered by blockade of α -receptors with phentolamine. The hypertension was not due to changes in blood gases or to release of agents from the kidneys or the adrenal glands; it was very sensitive to anesthetics and was abolished or aborted by midcollicular decerebration. Within hours after lesioning, the rats developed progressive congestive heart failure and died in shock, often in association with pulmonary edema. We concluded that the fulminating hypertension evoked by lesions of the nucleus tractus solitarius was due to the increased vasoconstriction caused by the augmented discharge of sympathetic nerves in response to central deafferentation of baroreceptor reflexes; the hypertension was mediated by α -receptors and depended on the integrity of structures lying above the midbrain.

2. Doba, N. and Reis, D.J.: The role of central and peripheral adrenergic mechanisms in neurogenic hypertension produced by brainstem lesions in rat. (NTS Hypertension). Circ. Res. 34: 293-301, 1974.

In this study we sought to determine some of the neurochemical mechanisms involved both peripherally and centrally in the mediation of baroreceptor reflexes.

Bilateral lesions of the nucleus tractus solitarius (NTS) in rats results in acute fulminating hypertension as a consequence of central deafferentation of baroreceptors. The hypertension is due to increased peripheral resistance with a decrease in cardiac output. The hypertension is blocked and cardiac output increased by phentolamine, trimethaphan (Arfonad) and reserpine but not by propranolol. Systemically administered 6-hydroxydopamine (6-OH-DA) does not alter the NTS hypertension if adrenal glands are intact.

Adrenalectomy, however, blocks the lesion-induced rise of blood pressure in 6-OH-DA treated animals. Intracisternal 6-OH-DA (600 µg/rat) lowers the concentration of norepinephrine only in spinal cord, and blocks the development of NTS hypertension. Local injection of 6-OH-DA into lateral hypothalamus does not affect the hypertension. Injection of 6-OH-DA into NTS results in mild, transient elevation of blood pressure. The results demonstrate that (a) NTS hypertension is due to increased sympathetic neural discharge; (b) in NTS hypertension adreno-medullary catecholamines are released which, when sympathetic terminals are destroyed, are sufficient to produce hypertension; (c) central noradrenergic neurons participate in the expression of NTS hypertension; and (d) baroreceptors may inhibit the release of adrenal catecholamines.

3. Amer, M.S., Doba, N. and Reis, D.J.: Changes in cyclic nucleotide metabolism in aorta and heart of neurogenically hypertensive rats: Possible trigger mechanism of hypertension. Proc. Nat. Acad. Sci. USA 72: 2135-2139, 1975.

Changes in cyclic nucleotide metabolism similar to those characteristic of the chronic forms of hypertension were observed in an acute neurogenic form of hypertension in rats produced by electrolytic lesions of the nucleus tractus solitarius. These changes that were evident two hours after the lesions were made included decreased cyclic AMP levels in the heart, increased cGMP:cAMP ratio, cAMP phosphodiesterase (3':5'-cAMP 5'-nucleotidohydrolase, EC 3.1.4.17) and guanylyl cyclase (GTP pyrophosphatase /cyclizing/, EC 4.6.1.2) activities in the aorta and decreased sensitivity of adenylyl cyclase (ATP pyrophosphate-lyase /cyclizing/, EC 4.6.1.1) in both the aorta and heart to stimulation by the β -adrenergic stimulant isoproterenol. These changes appear to depend on catecholamine release and are not due to mechanical distortion secondary to the increased arterial pressure. These studies provide biochemical support to the concept that the sympathetic nervous system may play a critical role in the initiation of the hypertensive syndrome and that chronic hypertension could result from the fixation of the biochemical effects of increased sympathetic activity.

4. Nathan, M.A. and Reis, D.J.: Fulminating arterial hypertension with pulmonary edema from release of adreno-medullary catecholamines after lesions of the anterior hypothalamus in the rat. Circ. Res. 37: 226-235, 1975.

Bilateral electrolytic lesions of the anterior hypothalamus in unrestrained rats resulted in the development, within 2 hours, of arterial hypertension, tachycardia, hyperthermia, and increased locomotor activity, often leading to pulmonary edema and death. Similar lesions in paralyzed, artificially ventilated rats produced comparable changes in

arterial blood pressure and body temperature with a similar time course. The arterial hypertension was a consequence of an increase in total peripheral resistance to 15% of control with a reduction in cardiac output to 49% of control. Arterial hypertension, elevated peripheral resistance and diminished cardiac output were reversed toward normal by α -receptor blockade with phentolamine (1 mg/kg, i.v.). Bilateral adrenalectomy, adrenal demedullation or adrenal denervation performed prior to lesion placement prevented the development of arterial hypertension and pulmonary edema as well as the changes in peripheral resistance, cardiac output, and body temperature. We conclude that arterial hypertension following lesions of the anterior hypothalamus is due to a neurally mediated increase in peripheral resistance initiated by the release of adrenal medullary catecholamines and that pulmonary edema is due to myocardial failure secondary to the ensuing ventricular overload. Structures originating in, or passing through, the anterior hypothalamus may exert selective control over the adrenal medulla independent of vasomotor neurons.

5. Nathan, M.A. and Reis, D.J. Chronic labile hypertension in cat by lesions of the nucleus tractus solitarii. *Circ. Res.* (in press, 1976).

Bilateral electrolytic lesions of the nucleus tractus solitarii (NTS) were made at the level of the obex in 7 cats. Within 1 hour after the lesions the mean arterial pressure (MAP) rose to a maximum of 144 mmHg (141% of control), and by 7 hours heart rate reached a high of 236 bpm (148% of control). The baroreceptor reflexes were abolished. After 24 hours the arterial pressure became extremely labile with variations of 80-100 mmHg observed. The lability occurred spontaneously and during behaviors that were self-initiated or elicited by environmental stimuli. The MAP in the lesion group was 144 mmHg (180% of control) during the day, and 96 mmHg (120% of control) at night. The lability, measured by the standard deviation, during the day in the lesion group was four times greater than in the control group and at night there were no differences. The heart rate of the lesion group was always higher than that of the control group but the lability of both groups was the same. We conclude that lesions of the NTS produced labile hypertension, probably by disinhibition of sympathetic activity through central interruption of the baroreceptor reflexes. The higher, more labile arterial pressures during the day may be caused by uninhibited increases in sympathetic activity elicited by environmental stimuli that are present during the day and absent at night. The daily variation of pressure may also be caused by somatomotor activity or by a daily rhythm of sympathetic activity which is unmasked by the lesions.

GROUP C: STUDIES ON BRAINSTEM MECHANISMS CONTROLLING
CIRCULATORY REFLEXES OTHER THAN ARTERIAL
BARORECEPTORS.

1. Doba, N. and Reis, D.J.: Localization within the lower brainstem of a receptive area mediating the pressor response to increased intracranial pressure (the Cushing response). Brain Res. 47: 487-491, 1972.

The extreme mobility of the brain within the cranium during normal postural shifts raises the possibility that intracerebral distortion/sensitive receptors which on stimulation evoke an elevation of the arterial pressure may participate in the regulation of orthostatic reflexes. The best known pressor response to distortion of the brainstem is the so-called "Cushing reflex". Recently, Hoff and Reis (Hoff, J.T. and Reis, D.J., Arch. Neurol. 23: 228, 1970) discovered that the Cushing reflex was due to stimulation of specific regions within the cerebrum and spinal cord and not due to distortion of intracranial extracerebral receptors. In that investigation it was established that in brain the receptive areas lie within the lower brainstem. By exploring the pons and medulla with a 1 mm probe it was discovered that the response could only be evoked by direct pressure over a paramedian strip lying along the floor of the IVth ventricle. The precise localization of the receptive areas within the substance of the brain beneath the pressure sensitive region, however, could not be identified.

In the present study we attempted to precisely identify the nuclear areas of the brain mediating the Cushing response. The studies were performed on cats anesthetized with chloralose, paralyzed with gallamine and artificially ventilated. Blood pressure and heart rate were measured by conventional methods. Regional blood flow was measured by an electromagnetic flow meter. The Cushing reflex was elicited in three different ways: (a) by inflating a 2 ml balloon inserted extradurally over the parietal cortex; (b) by local pressure applied to the exposed floor of the IVth ventricle by a stainless steel probe of 1.0 mm diameter attached to a force-displacement transducer by directly injecting 1-3 μ l of saline or artificial CSF for 1-2 seconds into the substance of the brainstem through a cannula mounted in an electrode carrier and also connected through a side-arm to a low pressure transducer. In other experiments the brainstem was electrically stimulated stereotaxically.

Diffusely increasing the intracranial pressure with a balloon or application of a probe to a restricted region paramedially on the floor of the IVth ventricle resulted in a graded stereotyped pattern of evoked cardiovascular activity. This consisted of increased systolic, diastolic, and mean arterial pressures, bradycardia, reduction

in blood flow and increased resistance in the femoral and mesenteric arteries, and a variable reduction of flow in the renal arterial bed. The threshold for the response was in the range of 165-200 mmHg either when intracranial pressure was increased diffusely by inflating the balloon or locally over the IVth ventricular floor by a probe. An identical cardiodynamic pattern could be produced by the rapid delivery of 1-3 μ l of artificial CSF from a cannula placed stereotactically in a restricted area contained within the rostral medulla and caudal pons lying deep to the probe-sensitive area. This area partially overlaps several nuclei in the lower brainstem reticular formation. A cardiovascular response of similar patterning could be evoked by punctate electrical stimulation restricted to the same region of the brainstem.

This study confirms and extends our previous studies which localized the receptive area in the brain for eliciting the Cushing response to a narrow paramedial strip lying along the floor of the IVth ventricle. It demonstrates that beneath the ventricular surface of the IVth ventricle a similar cardiovascular response can be evoked by small pressure transients in the order of 10-30 cm of water. The sensitive zone is geographically restricted and lies within the overlapping confines of several nuclei of the dorsolateral reticular formation. Since electrical stimulation in the same area can evoke the response it is likely that the Cushing response results from excitation rather than paralysis of neurons or fibers in the region. It is most likely that an adequate stimulus for the deformation within the receptive area is probably less than 10 cm of water places the threshold for activation of the Cushing response within the range of distorting forces (pressures) which might be naturally generated by vascular pulsations, shifts in the brain with change of posture, or extreme pressure stimuli resulting from increased G forces.

2. Doba, N. and Reis, D.J.: Central neural mechanisms mediating the pressor response to increased intracranial pressure (the Cushing response) (submitted to Brain Research).

The localization of regions in the brainstem mediating the elevation of arterial pressure and bradycardia elicited by an acute increase in intracranial pressure (the Cushing response) was determined in cats anesthetized with chloralose, paralyzed and artificially ventilated. In cat, the Cushing response, elicited by acutely increasing intracranial pressure by inflation of an intradural balloon, consists of an elevation of arterial pressure, bradycardia and increase in vascular resistance and decrease in blood flow in the femoral, renal and mesenteric arteries, and the increase in blood flow without change in resistance in the common carotid artery. Venous pressure is unchanged. In unparalyzed animals the response is associated with expiratory

apnea. Comparable circulatory changes could be elicited by transient distortion elicited by application of a 1 mm probe to a restricted area lying paramedially along the floor of the fourth ventricle with pressures of 150-300 mmHg. The Cushing response could be elicited by the rapid (less than 1 second) injection of 1-3 μ l of artificial CSF at pressures of 10-30 cm H₂O into a region of the pontomedullary tegmental reticular formation lying deep to probe-sensitive regions of the ventricular floor, and by electrical stimulation within the same sites. The bradycardia but not the rise of arterial pressure can be abolished by small bilateral lesions of the nucleus tractus solitarius which abolish baroreceptor reflexes from the periphery indicating that the bradycardia in the Cushing response is a secondary baroreceptor reflex. The Cushing response elicited by electrical stimulation of the brainstem is associated with the release of adrenal medullary catecholamines but in insufficient amounts to contribute to the rise of blood pressure. Lesions placed at the caudal extent of the distortion-sensitive regions in the medulla abolish the Cushing response when elicited electrically from rostral sites ipsilaterally. Bilaterally such lesions result in a fall of arterial pressure to levels similar to those elicited by spinal cord transection. We conclude, that the Cushing response is mediated by the distortion of neurons lying within the pontomedullary tegmentum which are exquisitely sensitive to distortion and possibly ischaemia. The pontotegmental region mediating the Cushing response may represent in part or in its entirety, the so-called tonic vasomotor area of the lower brainstem.

3. Kumada, M., Dampney, R.A.L. and Reis, D.J.: The trigeminal depressor response: A novel depressor response originating from the trigeminal system. Brain Res. (in press, 1976).

Electrical stimulation within the spinal trigeminal complex of anesthetized or decerebrated rabbit results in a fall of arterial pressure and bradycardia, a response termed the trigeminal depressor response (TDR). The TDR is associated with tachypnea at lower stimulus intensities and expiratory apnea at higher stimulus intensities and gastric hypermotility which is blocked by vagotomy. Within the spinal trigeminal complex, the TDR is regionally restricted; it is primarily elicited from the spinal trigeminal tract and nucleus caudalis; ipsilateral lesions of the spinal trigeminal complex near the obex abolish the TDR. Bradycardia and hypotension can be elicited by electrical stimulation of branches of all 3 divisions of the trigeminal nerve; such responses are abolished by ipsilateral lesions of the spinal trigeminal tract. The hypotension and bradycardia elicited from brain or nerve are elicited by low frequency stimulation (3-20 Hz); at frequencies over 50 Hz the response disappears and may become pressor; the responses from brain and nerve are graded to stimulus intensity up to 3-6 times the threshold

which, in brain is $\pm 10 \mu A$. The bradycardia of TDR is only abolished by vagotomy combined with β -adrenergic blockade and, thus, results from excitation of cardiac vagus and inhibition of cardiac sympathetic nerves. Abolition of baroreceptor reflexes from carotid sinus and aortic nerves by bilateral lesions of the nucleus tractus solitarii (NTS) fails to alter the TDR; conversely, lesions of the spinal trigeminal complex do not impair baroreceptor reflexes. Hypotension and bradycardia elicited by electrical stimulation of branches of the IXth nerve, other than baroreceptors, are impaired by lesions of the spinal trigeminal complex, but not NTS. We conclude that the TDR represents a heretofore unrecognized depressor response of unknown function integrated in the spinal trigeminal complex. It is distinct from arterial baroreceptor reflexes. It is reflexly elicited from widely distributed, but yet unidentified receptors in branches of the Vth nerve, and possibly, IXth and Xth cranial nerves.

4. Dampney, R.A.L., Kumada, M. and Reis, D.J.:
Cardiodynamic changes associated with cerebral ischaemic response in rabbit. (to be submitted to J. Physiol., London).

The cerebral ischaemic response was elicited in unanesthetized rabbits in which both vertebral arteries and one common carotid artery were occluded by transiently clamping the remaining common carotid artery for a period of up to 1 minute. The response is characterized by a large elevation in arterial blood pressure, bradycardia and apnea. The elevation in arterial pressure during the cerebral ischaemic response is associated with a marked fall in cardiac output and a very large increase in total peripheral resistance. Measurements of the blood flow changes in the femoral, mesenteric and renal beds demonstrated a highly reproducible pattern of vasoconstriction with the vasoconstriction being most intense in the renal bed and least in the femoral bed. The vasoconstriction is due primarily to an increase in sympathetic vasoconstrictor nerve activity. However, after sympathetic denervation of each of the beds, vasoconstriction still occurred in the renal and mesenteric beds, but the response in the femoral bed was reversed to a vasodilation due to the effect of released adrenomedullary catecholamines. In rabbits with denervated arterial baroreceptors, cerebral ischaemia excites both cardiac sympathetic and parasympathetic fibers with the net result usually being a small bradycardia. When the baroreceptors were intact, the bradycardia was much greater during ischaemia. The pattern of the cardiovascular and respiratory changes during the cerebral ischaemic response is very similar to that associated with oxygen conserving reflexes such as the diving and nasopharyngeal reflexes, and also to that which results from an increase in intracranial pressure (the Cushing response).

5. Dampney, R.A.L., Kumada, M. and Reis, D.J.: Localization of central neural regions mediating the cerebral ischaemic response in the rabbit. (to be submitted to J. Physiol., London).

The cardiovascular response to cerebral ischaemia persisted after brain transection through the rostral medulla (just rostral to the point of entry of the facial nerve) combined with transection of cranial nerves VII-XI, but was abolished by subsequent spinal cord section at C1. It therefore follows that the response is initiated by ischaemia acting directly on central nerve cells, and that the response is mediated by regions within the lower brainstem. Points were mapped within the lower brainstem which, when subjected to relatively low-intensity electrical stimulation (100 μ A amplitude, 50 Hz frequency, 0.5 msec pulse duration, 12 sec train) gave rise to a powerful hypertension similar to that elicited by cerebral ischaemia. Such points were found to be located in the dorsal reticular formation centered on the nucleus reticularis parvocellularis and extending from the middle portion of the inferior olive to the level of entry of the facial nerve. Within a certain range of intensities, stimulation of points within this responsive region resulted in a pattern of changes in blood pressure, renal and femoral conductances very similar to that associated with cerebral ischaemia. The changes in heart rate and respiration associated with cerebral ischaemia could not, however, be duplicated by electrical stimulation. Electrical lesions placed at the caudal limit of the responsive region resulted in a fall in blood pressure to levels observed in spinal animals. The lesions also abolished the pressor, but not the cardiac response to cerebral ischaemia. It is concluded, based on the stimulating and lesioning experiments, that this region is essential in the mediation of the vasomotor component of the cerebral ischaemic response, and for the maintenance of resting phasomotor tone.

6. Doba, N., Beresford, H.R. and Reis, D.J.: Changes in regional blood flow and cardiodynamics associated with electrically and chemically induced epilepsy in cat. Brain Res. 90: 115-132, 1975.

Changes in cardiodynamics and regional blood flow were examined in chronically prepared paralyzed cats during seizures induced electrically by transcerebral or direct cortical stimulation or by administration of flurothyl ether (Indoklon) or pentylenetetrazol (Metrazol). Transcerebral and chemical stimuli produced the greatest vascular responses. During seizures there was an abrupt elevation of arterial pressure unassociated with consistent changes in heart rate. Vascular resistance was increased in femoral, renal and mesenteric arteries with variable reductions in blood flow. Resistance was decreased and flow passively increased in the common carotid artery reflecting the loss

of cerebral autoregulation. Cardiac output was unchanged. With seizures associated with large elevations of arterial pressure, the central venous and left ventricular end-diastolic pressures were markedly increased indicating incipient congestive failure. The pressor response was blocked by α -adrenergic blockade with phentolamine. Increased regional vascular resistance was abolished by regional sympathectomy. While either adrenalectomy or treatment with 6-hydroxydopamine alone failed to abolish the pressor response, combined, they did. Such treatment unmasked an atropine-sensitive bradycardia. The pressor response with seizures is a consequence of increased vascular resistance in viscera and muscles due to widespread activation of sympathetic neurons and release of adrenomedullary catecholamines. Co-activation of cardiovagal and cardiosympathetic neurons may underlie some associated arrhythmias. Cardiovascular events may serve, by redistribution of the cardiac output, to assure increased availability of oxygen and nutrients to brain to meet the metabolic demands of convulsions.

GROUP D: OTHER RELATED STUDIES AND REVIEWS.

1. Nathan, M.A. and Reis, D.J.: Hypoxemia, atelectasis, and the elevation of arterial pressure and heart rate in paralyzed artificially ventilated rat. *Life Sci.* 16: 1103-1120, 1975.

Rats prepared while anesthetized with halothane, ether or pentobarbital, subsequently paralyzed with curare, and maintained with or without anesthetic, by artificial ventilation with room air, are hypoxemic in association with elevated arterial pressures and heart rates. The hypoxemia can occur with normal P_{aCO_2} , is associated with a marked increase in the alveolar-arterial P_{O_2} difference, and is not reversed by hyperventilation or hyperinflation. The lungs, visualized directly through a thoracotomy during artificial ventilation, are segmentally collapsed and at postmortem demonstrate focal and diffuse signs of atelectasis. Hypoxemia and an elevation of the alveolar-arterial P_{O_2} difference occur within 20 minutes. We conclude that anesthetized rats develop atelectasis soon after the onset of anesthesia. The atelectasis and resultant hypoxemia persist during subsequent paralysis despite an adequate minute volume and absence of anesthesia. Despite atelectasis, blood gases, arterial pressures and heart rates may be maintained near normal values by ventilation of paralyzed rats with 50% O_2 and 50% N_2 .

2. Reis, D.J., Nathan, M.A. and Doba, N.: Two specific brainstem systems which regulate the blood pressure. *Clin. and Exp. Pharmacol. Physiol.*, Suppl 2: 179-183, 1975.

Two systems of brainstem involved in the regulation of arterial pressure, the nucleus tractus solitarii (NTS)

and a dorsal region of the pontomedullary tegmentum have been objects of investigation in our laboratory. The NTS mediates baroreceptor reflexes in brain. Lesions in rat result in development of fulminating arterial hypertension as a consequence of abolition of baroreceptor reflexes centrally and release of sympathetic vasomotor neurons from inhibition. In cat, NTS lesions result in the development over one week of labile neurogenic hypertension which is sustained. Neuronal systems of the upper brainstem as well as neuronal systems which store, synthesize and release the catecholamine neurotransmitters are involved in NTS-mediated regulation of the blood pressure. A pressor system in the pontomedullary tegmentum consists of neurons which respond to focal distortion and mediate the pressor response to increased intracranial pressure (the Cushing reflex). Lesions transecting the caudal pathway of the tegmental pressor system results in a fall of blood pressure to levels resulting from transection of the spinal cord. This tegmental pressor system may correspond to the tonic vasomotor neuronal system of the lower brainstem whose integrity is necessary for the maintenance of normal levels of blood pressure.

3. Reis, D.J. Nathan, M.A. and Doba, N.: Two models of arterial hypertension in rats produced by lesions of inhibitory areas of brain. In: The Nervous System in Arterial Hypertension, eds., Julius, S., Esler, M.D., Charles C. Thomas (Publisher), Springfield, IL, pp 119-150, 1976.

Two models of acute fulminating arterial hypertension can be produced in rats by brain lesions which destroy central inhibitory pathways. The first, NTS-hypertension, is a consequence of central deafferentation of baroreceptors made by lesions placed at the site of their termination in the brain in NTS. This mode of hypertension develops immediately following recovery from anesthesia and is characterized by increased total peripheral resistance and an associated fall of cardiac output. It is mediated entirely by increased activity of sympathetic vasomotor fibers. The central pathways mediating the hypertension are not known, but depend upon the integrity of structures lying above the midbrain and also of descending noradrenergic tracts. Within 2 hours following the onset of increased sympathetic vasomotor activity changes can be observed within the cyclic nucleotide metabolism of blood vessels and heart with a decrease of cyclic AMP and an increase of cyclic GMP occurring in the aorta, and a decrease of cyclic AMP occurring in the heart. The alterations of cyclic AMP are the result from increased synthesis. Such changes in cyclic nucleotides may be related to triggering mechanisms which lead to enduring changes in the metabolism of blood vessels which may serve to fix the hypertension.

The second syndrome of hypertension can be produced by lesions of the anteromedial hypothalamus (AH-hypertension). The hypertension in this instance develops after a latency of 60-90 minutes, is associated with markedly increased motor activity, and hyperthermia, and generalized sympathetic arousal. It is abolished by bilateral adrenalectomy, by adrenal demedullation, and selective adrenal denervation. It therefore appears to be the consequence of interruption of fibers originating in or passing through the anterior hypothalamus which selectively inhibit the release of adrenomedullary catecholamines.

Our findings indicate that selective interruption of central systems modulating sympathetic activity can lead to arterial hypertension. Such models of acute hypertension in rats may be useful in further analyzing central neural mechanisms governing the discharge of the CNS, and which might be impaired in the human counterpart of the disease.

4. Reis, D.J. and Doba, N.: The central nervous system and neurogenic hypertension. Prog. in Cardiovasc. Dis. 17: 51-71, 1974.

It is evident from studies on animals that manipulation of the central neural mechanisms governing the discharge of sympathetic neurons can lead to prolonged and even lethal arterial hypertension. Such neurogenic hypertension can be produced by: (a) interruption at peripheral or central sites of reflexes tonically inhibitory to the sympathetic nervous system; (b) elevation of the intracranial pressure, which leads to distortion by stretch/pressure of specific neurons of the brainstem and spinal cord; (c) direct distortion of sensitive regions of the lower brainstem; (d) cerebral ischaemia and/or hypoxia; (e) electrical stimulation of specific neural systems that excite sympathetic neurons; (f) selective brain lesions; (g) chronic stress; or (h) learned responses. Moreover, there is increasing evidence that biochemically specific pathways in the brain, notably those that synthesize, store, and release the neurotransmitter norepinephrine may be critical for the expression of increased sympathetic tone. It may be through these sites that agents synthesized peripherally, such as angiotensin, act in regulating vasomotor tone systemically. Indeed, it may also be at these control sites that several of the pharmacologically active agents that are clinically efficacious in controlling hypertension in man may act. Finally, there is increasing evidence that the metabolism of cyclic nucleotides, which are in part related to receptive elements within blood vessels, may be altered by sympathetic discharge, particularly when prolonged, leading to enduring changes in vascular resistance that ultimately may lead to a fixed hypertensive state.

III. PUBLICATIONS OF RESEARCH SUPPORTED BY NASA
(NGR-33-010-179) 1972-1976

1. Sheridan, C. and Reis, D.J.: Effects of cerebellar ablation on regional distribution of cardiac output. Brain Res. 45: 260-265, 1972.
2. Doba, N. and Reis, D.J.: Localization within the lower brainstem of a receptive area mediating the pressor response to increased intracranial pressure (the Cushing response). Brain Res. 47: 487-491, 1972.
3. Doba, N. and Reis, D.J.: Changes in regional blood flow and cardiodynamics evoked by electrical stimulation of the fastigial nucleus in cat: similarity to orthostatic reflexes. J. Physiol. 227: 729-748, 1972.
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9. Nathan, M.A. and Reis, D.J.: Hypoxemia, atelectasis, and the elevation of arterial pressure and heart rate in paralyzed artificially ventilated rats. Life Sci. 16: 1103-1120, 1975.
10. Doba, N., Beresford, H.R. and Reis, D.J.: Changes in regional blood flow and cardiodynamics associated with electrically and chemically induced epilepsy in cat. Brain Res. 90: 115-132, 1975.

11. Reis, D.J., Nathan, M.A. and Doba, N.: Two specific brainstem systems which regulate the blood pressure. Clin. and Exp. Pharmacol. Physiol. Suppl 2: 179-183, 1975.
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15. Kumada, M., Dampney, R.A.L. and Reis, D.J.: The trigeminal depressor response: A novel vasodepressor response originating from the trigeminal system. Brain Res. 1976. (in press).
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Effects of cerebellar ablation on regional distribution of cardiac output in cat

It has long been known that the cerebellum may *phasically* influence the circulation. Electrical stimulation of cerebellar cortex or deep nuclei has evoked transient changes in blood pressure, heart rate, the distribution of organ blood flow, and the sensitivity of baroreceptor and other cardiovascular reflexes¹⁻⁵. It remains to be determined if the cerebellum regulates the circulation *tonically*. While acute total cerebellar ablation does not alter resting systemic blood pressure or heart rate, it augments baroreceptor reflexes⁶, suggesting some tonic action by cerebellum on the circulation. One possibility is that the cerebellum may function to regulate the distribution of the cardiac output since widespread shifts of organ blood flow may occur unreflected by changes in the systemic blood pressure.

In this study we have investigated the effects of total cerebellar ablation in cat, both acutely and chronically, on the distribution of nutrient (*i.e.* non-hunt) organ blood flow. Because of the important role of the cerebellum in the regulation of posture and movement, attention has been paid to the effects of cerebellar ablation on the selective distribution of blood flow to red and white skeletal muscle whose basal blood flow differs at rest and is differentially regulated in sleep, quiet waking, and excited behavior^{7,8}.

The studies were performed on 14 mature cats of both sexes, weighing between 2.5 and 4.5 kg. Five animals served as controls. Under pentobarbital anesthesia (40 mg/kg), the cerebellums of 9 cats were removed by suction under sterile conditions. The wound was closed in layers and penicillin (20,000 U, i.m.) administered. Because of the severe deficit in motility postoperatively, the animals were fed an artificial nutrient (Similac) through a gastric tube until they were killed. No cat lost more than 8% of his original body weight.

The fractional distribution of blood flow was measured in 5 cats 3 days after cerebellectomy (subacute group) and in 4 cats 28 days after surgery (chronic group). The extent of the lesions was confirmed postmortem and in all cases cerebellar ablation was complete.

Blood flow distribution to skeletal muscle and viscera of unanesthetized cats was measured by modification^{7,8} of the isotope dilution method of Sapirstein¹⁰ using ⁸⁶Rb as an indicator. In summary, the method is based on the fact that Rb, like K, will rapidly distribute itself in the intracellular compartment of most tissues (a notable exception being the CNS) and remain at a constant concentration for up to 2 min. Since most of the injected ⁸⁶Rb is extracted in one circulation¹⁰, the radioactivity of an organ is proportional to the percentage of the cardiac output reaching that tissue. The ratio of organ activity to injected activity is termed the fractional flow (FF) and calculated:

$$FF = \frac{\text{organ activity (counts/min/g)} \times 100}{\text{total injected activity (counts/min)}}$$

If the cardiac output is known, it is possible to measure organ blood flow by the calculation

$$\text{absolute flow (ml/min/100 g)} = \text{FF} \times \text{cardiac output (ml/min)}.$$

In this study the cardiac output can be considered a constant since we are interested in the relative distribution of blood flow to different types of tissue measured simultaneously in the same animal. Thus, comparison of fractional flow to different tissues between experimental and control groups will serve by itself to indicate the effects of cerebellar ablation on distribution of nutrient blood flows^{7,8}.

At 2 or 27 days after cerebellectomy, under halothane anesthesia, a polyethylene cannula was threaded down the right external jugular vein until its tip was estimated to lie just above the right atrium. The cannulas were fixed in deep tissue and brought out posteriorly through a stab wound in the neck. The cannula was flushed with a saline solution and plugged with a trochar. Twenty-four hours later, 4 or 5 ml of an isotonic solution of ⁸⁶RbCl (specific activity 5 μ Ci/ml) was injected through the cannula and immediately flushed with 0.5 ml of normal saline. This was followed 1 min later by a bolus of saturated KCl solution which caused instantaneous death from cardiac arrest. Care was taken to ensure that all animals were recumbent before the injections were given to eliminate any effect of posture on the blood flow.

Samples of tissue of approximately equal weight were removed, blotted dry, weighed, and then counted in an automated gamma-well type scintillation counter. The results were expressed as counts/min/g of tissue. Skeletal muscles were classified as white or red on the basis of the criteria of color, myoglobin concentration, capillary density, and contraction time, as described in detail by Reis and Wooten⁸. FF values were expressed for individual muscles (Table 1) or grouped red and white muscles (Fig. 1)⁷.

Three days after cerebellar ablation all cats demonstrated the profound deficits of motor performance that have been described by others³ including nystagmus, intermittent extensor rigidity, opisthotonos, decomposition of movement, and hypermetria. They were unable to stand or feed themselves. Although motor function gradually improved in the subacute group it had not completely returned to normal by the 28th day. At that time although all animals could rise to a standing position unassisted they could only take a few ataxic steps without support and stand for longer periods by leaning against the wall of the cage. Opisthotonos and nystagmus were not present. These changes parallel the experience of others with the evolution of motor compensation during recovery from total cerebellar ablation in cat.

Changes in the regional distribution of the cardiac output to various muscles and selected viscera at 3 and at 28 days after total cerebellectomy are shown in Table I and are in agreement with previous data from this laboratory^{7,8}. The only significant changes noted are a decrease in the FF to selected or grouped (Fig. 1) red skeletal muscles at 3 days. As a consequence of the reduced FF to red muscle the 3:1 ratio of red to white muscle flow characteristic of quiet waking⁷ approached unity (Fig. 1). The decrease in FF to red muscles is transient since by 28 days, with the exception of the medial head of the triceps, the changes in FF no longer differ significantly from

TABLE I

CHANGES IN THE FRACTIONAL DISTRIBUTION OF CARDIAC OUTPUT TO SELECTED RED AND WHITE SKELETAL MUSCLES AND VISCERA AT 3 DAYS (SUBACUTE) AND 28 DAYS (CHRONIC) AFTER TOTAL CEREBELLECTOMY

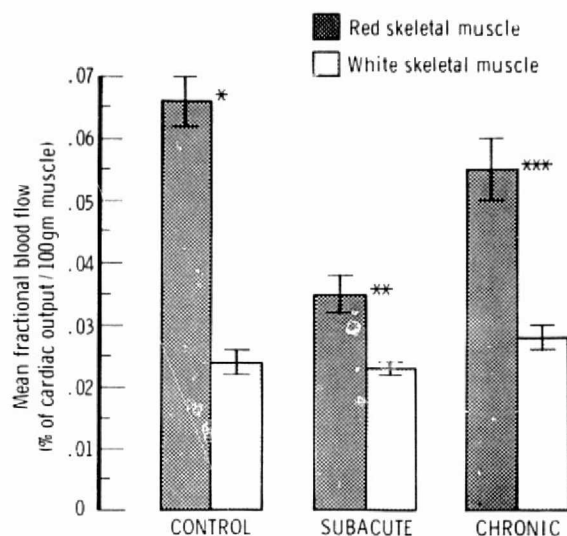
Muscle		Control		Subacute		Chronic	
		Color*	FF** (5)	FF** (5)	P***	FF**	P***
Triceps (short, med.)	R		0.086 ± 0.008 (5)	0.042 ± 0.003 (5)	P < 0.001	0.060 ± 0.012 (4)	P < 0.05
Diaphragm	R		0.072 ± 0.007 (5)	0.066 ± 0.004 (5)	NS	0.080 ± 0.010 (4)	NS
Soleus	R		0.066 ± 0.006 (5)	0.016 ± 0.004 (5)	P < 0.001	0.042 ± 0.008 (4)	NS
Crureus	R		0.069 ± 0.006 (5)	0.023 ± 0.003 (5)	P < 0.001	0.046 ± 0.011 (4)	NS
Supraspinatus (deep)	R		0.041 ± 0.008 (5)	0.035 ± 0.005 (5)	NS	0.052 ± 0.010 (4)	NS
Semitendinosus	W		0.033 ± 0.004 (5)	0.019 ± 0.006 (5)	NS	0.040 ± 0.011 (4)	NS
Ext. carpi radialis	W		0.033 ± 0.007 (5)	0.029 ± 0.003 (5)	NS	0.027 ± 0.003 (4)	NS
External intercostal	W		0.032 ± 0.005 (5)	0.030 ± 0.005 (5)	NS	0.049 ± 0.011 (4)	NS
Triceps (long, int.)	W		0.029 ± 0.005 (5)	0.037 ± 0.006 (5)	NS	0.034 ± 0.004 (4)	NS
Gluteus maximus	W		0.028 ± 0.004 (5)	0.022 ± 0.004 (5)	NS	0.043 ± 0.011 (4)	NS
Biceps	W		0.024 ± 0.009 (5)	0.026 ± 0.004 (5)	NS	0.022 ± 0.004 (4)	NS
Tibialis ant.	W		0.024 ± 0.002 (5)	0.028 ± 0.010 (5)	NS	0.021 ± 0.006 (4)	NS
Gastrocnemius (med)	W		0.023 ± 0.003 (5)	0.018 ± 0.003 (5)	NS	0.029 ± 0.007 (4)	NS
Supraspinatus (sup)	W		0.022 ± 0.002 (5)	0.023 ± 0.001 (5)	NS	0.045 ± 0.010 (4)	NS
Adductor femoris	W		0.021 ± 0.005 (5)	0.027 ± 0.011 (5)	NS	0.027 ± 0.012 (4)	NS
Semimembranosus	W		0.016 ± 0.005 (5)	0.021 ± 0.005 (5)	NS	0.030 ± 0.009 (4)	NS
Vastus lateralis	W		0.013 ± 0.004 (5)	0.015 ± 0.002 (5)	NS	0.026 ± 0.010 (4)	NS
Lateral rectus (extraocular muscle)			0.095 ± 0.026 (5)	0.072 ± 0.009 (5)	NS	0.106 ± 0.014 (4)	NS
Left ventricle			0.302 ± 0.015 (5)	0.297 ± 0.011 (5)	NS	0.287 ± 0.015 (4)	NS
Right ventricle			0.177 ± 0.045 (5)	0.186 ± 0.023 (5)	NS	0.172 ± 0.012 (4)	NS

Organ					
Esophagus	0.038 ± 0.002 (5)	0.041 ± 0.007 (5)	NS	0.034 ± 0.007 (4)	NS
Stomach	0.071 ± 0.009 (5)	0.077 ± 0.005 (5)	NS	0.090 ± 0.023 (4)	NS
Jejunum	0.082 ± 0.012 (5)	0.109 ± 0.024 (5)	NS	0.062 ± 0.013 (4)	NS
Ileum	0.090 ± 0.003 (5)	0.085 ± 0.036 (5)	NS	0.077 ± 0.015 (4)	NS
Transverse colon	0.079 ± 0.005 (5)	0.067 ± 0.009 (5)	NS	0.075 ± 0.024 (4)	NS
Thyroid	0.098 ± 0.020 (5)	0.102 ± 0.029 (5)	NS	0.081 ± 0.017 (4)	NS
Adrenals	0.106 ± 0.018 (5)	0.159 ± 0.011 (5)	NS	0.126 ± 0.011 (4)	NS
Pancreas	0.130 ± 0.005 (5)	0.136 ± 0.020 (5)	NS	0.151 ± 0.033 (4)	NS
Kidney medulla	0.336 ± 0.018 (5)	0.277 ± 0.008 (5)	NS	0.286 ± 0.021 (4)	NS
Kidney cortex	0.410 ± 0.063 (5)	0.470 ± 0.017 (5)	NS	0.305 ± 0.053 (4)	NS
Spleen	0.079 ± 0.006 (5)	0.113 ± 0.015 (5)	NS	0.089 ± 0.017 (4)	NS

* Color is red (R) or white (W) as defined by Reis and Wooten⁸.

** FF expressed as % of cardiac output/100 g tissue ± S.E.M. (n).

*** Significance of difference from control ($P < 0.05$) by Student's *t*-test (NS = not significant).



* Differs from white control ($p < .001$)

** Differs from white subacute ($p < .01$),
differs from red control ($p < .001$)

*** Differs from white chronic ($p < .001$),
difference from red control not significant

Fig. 1. Changes in fractional distribution of cardiac output (mean \pm S.E.M.) to grouped red and white skeletal muscle 3 days (subacute) and 28 days (chronic) after total cerebellectomy in cat. Red muscles are designated as R and white muscles as W as in Table I (according to criteria of Reis and Wooten⁸).

control. Since the changes in flow to the medial triceps in chronic animals are returning to normal it is probable that the reduced flow at 28 days only reflects a slower rate of recovery.

This study therefore demonstrates that despite the profound abnormalities in motor activity which result from cerebellectomy, changes in the regional distribution of organ nutrient blood flow are relatively small. The only effect of total cerebellar ablation is a selective decrease in the FF to red skeletal muscles of the limbs. The decrease is transient and, in the main, returns to normal by 28 days after surgery.

The redistribution of blood flow away from red muscles during the acute stage of disability does not appear to be directly related to the profound postural impairment reflecting a decrease in the activity of red limb muscles since: (a) all measurements of FF were done with the animals lying down and (b) the difference in flow between red and white skeletal muscles does not depend upon motor activity since it persists under anesthesia¹⁰ and when the motor nerves are sectioned¹¹. Indeed the only time in which red muscle blood flow is reduced in a comparable manner is during the rapid eye movement (REM) phase of sleep⁷. The changes in red muscle blood flow following cerebellar ablation therefore appear to represent the effect of withdrawal of some cerebellar influence on the circulation. However the parallel recovery of

motor function and blood flow to red muscle suggests a close functional link between the compensatory mechanisms in autonomic and somatic motor activities.

The finding that the cerebellum may tonically regulate the distribution of blood flow to red skeletal muscle is not surprising in view of the important role of the cerebellum in governing posture and the selective participation of red skeletal muscle in this activity. The failure to observe changes in blood flow in white skeletal muscle after cerebellar lesion may be related to the fact that animals without a cerebellum can still move phasically (albeit not in a coordinated manner) and that at rest blood flow in white muscle is nearer to minimal values.

It remains to be determined if the effects of cerebellar ablation are due to removal of cerebellar cortex, deep nuclei or both areas. Changes in muscle blood flow can be elicited by electrical stimulation from restricted sites of cortex (anterior vermis⁴) and also from fastigial nucleus^{1,2}. However, since cortical stimulation tends to increase muscle flow⁴ while fastigial stimulation always reduces it^{1,2}, the deficit of cerebellar ablation would appear to result preponderantly from a functional loss of an action of the cerebellar cortex on the circulation.

The authors thank Dr. G. F. Wooten for useful advice.

The research was supported by grants from the NIH (NB 04876), the National Aeronautics and Space Administration (NGR-33-010-179), and the Harris Foundation.

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(Accepted July 17th, 1972)

Short Communications

Localization within the lower brainstem of a receptive area mediating the pressor response to increased intracranial pressure (the Cushing response)

An acute increase in the intracranial pressure has long been known to elicit a rise of systemic blood pressure and a slowing of the heart^{4,9}. The response is commonly referred to in the clinical literature as the 'Cushing reflex' or 'Cushing response' after the neurosurgeon who first established its quantitative relationship to the intracranial pressure⁴.

The nature and localization of the 'receptors' initiating the Cushing response have only been partially identified. Hoff and Reis⁹ established that, in brain, the receptive areas lie within the lower brainstem. By exploring the pons and medulla with a 1 mm probe they discovered that the response could only be evoked by direct pressure over a paramedian strip lying along the floor of the fourth ventricle. The precise localization of the receptive areas within the substance of the brain beneath the pressure sensitive region, however, could not be identified.

In the present study we have therefore attempted to precisely identify the nuclear areas of the brain mediating the Cushing response. We shall demonstrate that the cardiodynamic features of the Cushing response^{7,11} can be evoked by tissue distortion elicited within the substances of brain by a microinjection technique, or by electrical stimulation of a highly restricted area within the rostral medulla and caudal pons.

These studies were performed on 53 cats anesthetized with 1% α -chloralose (50 mg/kg, i.v.), paralyzed with gallamine triethiodide (10 mg/kg) and artificially ventilated with maintenance of end-tidal CO₂ between 2-3%. Methods for recording cardiovascular activity, stimulating brain, and locating electrodes have been detailed elsewhere^{3,6,9,12}. Aortic blood pressure was measured from the descending aorta via the femoral artery; the heart rate was computed from the pulse wave by a cardiometer. Blood flow was measured by an electromagnetic flow meter. Flow probes were placed over appropriate arteries with zero flow being repeatedly checked during the experiment by occlusion. Intracranial pressure was measured by a catheter placed in the cerebellopontine angle through an occipital craniotomy tightly sealed with dental acrylic⁹. All outputs from transducers were displayed on channels of a polygraph.

The Cushing response was elicited in 3 different ways: (a) by inflating a 2 ml balloon inserted extradurally over the parietal cortex⁹; (b) by local pressure applied to the exposed floor of the IVth ventricle by a stainless steel probe of 1.0 mm diameter attached to a force-displacement transducer;⁹ (c) by directly injecting 1-3 μ l of saline or artificial CSF over 1-2 sec into the brainstem through a cannula of 30-gauge stainless steel hypodermic tubing mounted in an electrode carrier connected to a 50 μ l Hamilton syringe with a hand operated dispenser (Hamilton model PB600) and connected through a side-arm to a low pressure transducer. The sites of injection were

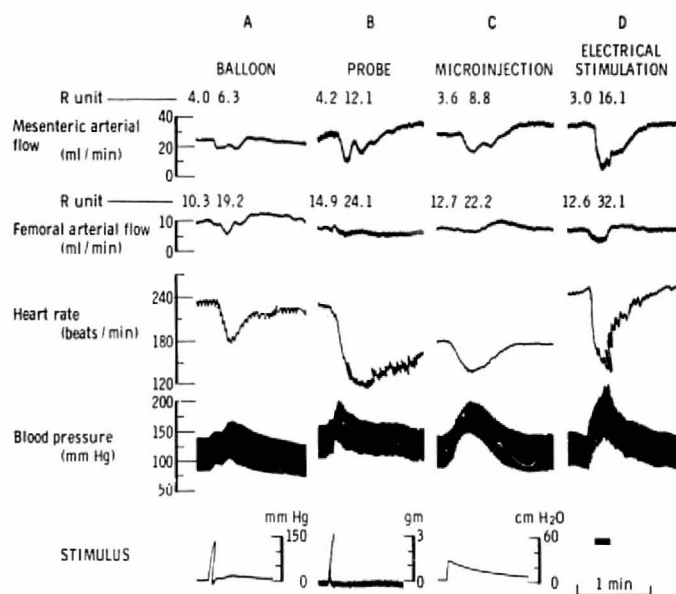


Fig. 1. The Cushing response evoked by: (A) diffusely increasing intracranial pressure by inflation of an epidural balloon (intracranial pressure indicated on bottom channel); (B) probing a paramedial site on the floor of the IVth ventricle with a 1 mm probe; (C) microinjection of 3 μ l of artificial CSF into the dorsal pontine tegmentum; (D) electrical stimulation of the same region as (C) with a square wave stimulus train of 12 sec (pulse duration 0.1 msec, frequency 50 c/sec, intensity 0.05 mA). Paralyzed, anesthetized cat. Blood flow and resistance (R) in the mesenteric (upper channel) and femoral arteries (2nd channel); heart rate (3rd channel); blood pressure (4th channel).

identified by delivering fast green dye through the cannula at the end of the experiment and subsequently examining the brain.

Electrical stimulation of the brainstem was performed stereotactically through teflon-coated stainless steel wires bared at the tip for 0.3 mm. The stimulus consisted of a 12 sec train of square wave pulses of 0.1 msec duration, delivered at a frequency of 50 c/sec. At the end of the experiment the animal was perfused with a mixture of potassium, ferri- and ferro-cyanide (in order to identify the tip of the stimulating electrode) followed by 10% formalin. Brains were cut frozen and appropriate sections mounted and stained for cells or fibers.

In confirmation of earlier studies^{9,10} diffusely increasing the intracranial pressure with a balloon (Fig. 1A) or application of a probe to a restricted region paramedially on the floor of the IVth ventricle (Fig. 1B), resulted in a graded and stereotyped pattern of evoked cardiovascular activity. This consisted of an increase in systolic, diastolic, and mean arterial pressures, bradycardia, reduction in blood flow and increase in resistance in the femoral and mesenteric arteries, and a variable reduction of flow in the renal arterial bed. The threshold for the response was in the range of 165–200 mm Hg either when intracranial pressure was increased diffusely by inflating the extradural balloon or locally over the IVth ventricular floor by a probe⁹. The relative magnitude of the changes in regional flow, systemic blood

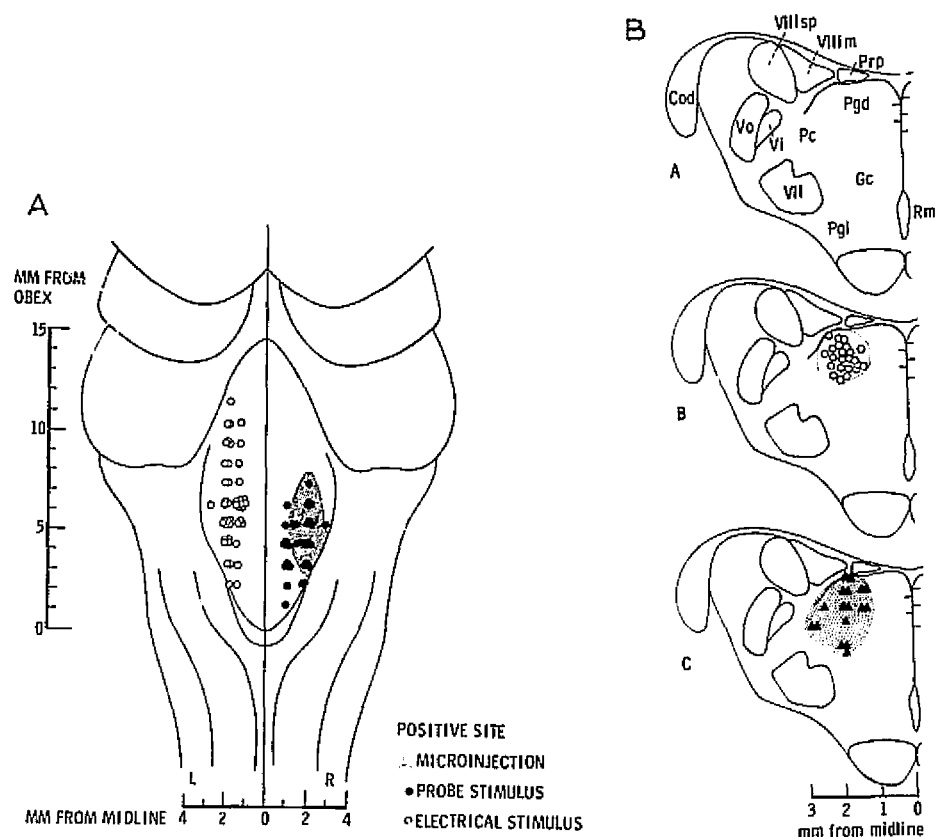


Fig. 2. A, Dorsal view of cat brainstem showing on the right side of the drawing the positive sites from which the Cushing response was evoked by local probe pressure (closed circles); the region projected to the surface of the brain from which microinjection evoked a similar response (stippled area); and, on the left side of the drawing, the dorsal projection of the electrode tracks from which the response was evoked by electrical stimulation (open circles). Scales on ordinate and abscissa represent distance from obex in mm. B, Representative cross section of brainstem of cat at the level of the facial nucleus. A, Control. Abbreviations (modified from Taber¹³): Cod, nucleus cochlearis dorsalis; Gc, nucleus gigantocellularis; Pc, nucleus parvocellularis; Pgd, nucleus paragigantocellularis dorsalis; Pgl, nucleus paragigantocellularis lateralis; Prp, nucleus praepositus hypoglossi; Rm, nucleus raphé magnus; Vo, nucleus tractus spinalis oralis; VII, nucleus nervi facialis; VIIIm, nucleus vestibularis medialis; VIIIsp, nucleus vestibularis spinalis. B, Localization of the lowest threshold points (open circles) from which electrical stimulation evokes the cardiovascular changes characteristic of the Cushing response from 23 electrode tracks. C, Localization of sites (closed triangles) from which the Cushing response was evoked by microinjection of 1-3 μ l of artificial CSF in 17 experiments. The stippled area represents the horizontal extent of the zone depicted in its rostrocaudal and lateral extent in Fig. 2a.

pressure, and heart rate varied between different animals. However, in repeated trials in any single animal they remained relatively constant.

An identical cardiodynamic pattern could be produced by the rapid delivery of 1-3 μ l of artificial CSF from a cannula placed stereotactically within a restricted area contained within the rostral medulla and the caudal pons lying deep to the probe-

sensitive area on the surface of the IVth ventricle (Figs. 1C, 2A and B). This area partially overlapped several nuclei of this portion of the lower brainstem^{1,13} including the more dorsal portion of the nucleus reticularis gigantocellularis (Fig. 2B, Gc), the nucleus reticularis parvocellularis (Fig. 2B, Pc), the nucleus reticularis pontis caudalis of Brodal¹ and included a nucleus designated nucleus paragigantocellularis dorsalis by Taber¹³ (Fig. 2B, Pgd).

The adequate stimulus appeared to be a transient distortion of tissue within the critical area because: (a) injection of a larger volume in adjacent areas failed to produce a response; (b) the response could not be evoked when the fluid was delivered slowly (over 5–20 sec); and (c) the response was independent of the chemical composition of the fluid or its pH. The threshold for the response ranged from 10 to 30 cm H₂O (5–10% of the threshold pressure required by application of a probe to the floor of the IVth ventricle⁹).

A cardiovascular response of similar patterning* could also be evoked by punctate electrical stimulation restricted to the same region of the brainstem (Figs. 1D and 2A, B). This area was identified by carefully mapping stimulus current–response contours along electrode tracks and identifying the points of lowest threshold³.

The present investigation therefore confirms and extends the observations of Hoff and Reis⁹ which localized the receptive area in brain for eliciting the Cushing response to a narrow paramedial strip lying along the floor of the IVth ventricle. It demonstrates that beneath the ventricular surface a similar cardiovascular response can be evoked by small pressure transients in the order of 10–30 cm H₂O. The sensitive zone is geographically restricted and lies within the overlapping confines of several nuclei of the dorsal reticular formation. Since electrical stimulation in the same area can also evoke the response, it is likely that the Cushing response results from excitation rather than 'paralysis' of neurons or fibers in the region.

The nature of the stimulus evoking the Cushing response and the identity of the receptive elements in the brainstem remain elusive. It is unlikely, in view of its brief latency, its low threshold, and its dependence on a rapid transient of pressure, that the response is triggered by local ischemia, as others have suggested⁵. Rather we favor the view that the adequate stimulus is stretch of receptive elements by tissue distortion, a view proposed by others¹⁰. The absence of any morphologically distinct 'receptors' in this region however raises the as yet unanswered question as to what is stimulated by the local tissue deformation, neurons or fibers? However, the findings raise the intriguing possibility that some brainstem neurons may be particularly sensitive to deformation, possibly in an analogous manner to the stretch-sensitive neurons of invertebrates⁸.

The question of the biological significance of the response remains to be answered. The fact that the threshold for deformation within the receptive area is in a range of 10–30 cm of H₂O (probably an underestimation of the threshold at the

* It should be emphasized that while electrical stimulation of a wide area of the lower brainstem produces pressor responses, as noted previously by others², the characteristic pattern of the Cushing response, primarily hypertension with bradycardia, was limited to the area indicated in Fig. 2A and B.

receptive site) now places the threshold for activation of the Cushing response in the medulla within the range of distorting forces (pressures) which might be naturally generated by vascular pulsations. This suggests that the neuronal areas mediating the Cushing response may serve some other, as yet to be identified, function in central cardiovascular control.

This study was supported by Grants from the NIH (NS 04876) and the National Aeronautics and Space Administration (NGR-33-010-179).

Nancy Shieh and Jana Iglich provided excellent technical support.

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(Accepted September 6th, 1972)

CHANGES IN REGIONAL BLOOD FLOW AND
CARDIODYNAMICS EVOKED BY ELECTRICAL STIMULATION
OF THE FASTIGIAL NUCLEUS IN THE CAT AND THEIR
SIMILARITY TO ORTHOSTATIC REFLEXES

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(Received 22 May 1972)

SUMMARY

1. Changes in regional blood flow and cardiodynamics were measured in anaesthetized paralysed cats during electrical stimulation of the rostral fastigial nucleus.

2. Fastigial stimulation results in a graded, highly reproducible and stereotyped cardiovascular response characterized by (a) increased systolic, diastolic and mean arterial pressures without changes in central venous or occluded vein pressure, (b) decreased blood flow and increased vascular resistance in the axillary, renal, femoral and mesenteric arteries, increased flow without any change in vascular resistance in the common carotid artery, and increase in total peripheral resistance, (c) a small increase in heart rate and myocardial contractile force, decrease in calculated stroke volume, and no change in the cardiac output.

3. Changes in regional arterial flow were abolished by transection of sympathetic nerves or blockade of α -adrenergic receptors by systemic administration of phentolamine.

4. Changes in heart rate and myocardial contractility were abolished by stellate ganglionectomy or blockade of β -adrenergic receptors by propranolol.

5. No changes in pupillary diameter or retraction of the nictitating membrane were seen during fastigial stimulation with stimuli producing substantial changes in blood pressure.

6. The fastigial pressor response represents a highly reproducible, stereotyped, graded, and differentiated pattern of activation of sympathetic preganglionic neurones.

7. The pattern of cardiovascular effects of fastigial stimulation simulates the compensatory (orthostatic) reflex response to maintenance of an upright posture.

8. Fastigial stimulation appears to excite the neural network subserving orthostatic reflexes.

INTRODUCTION

Electrical stimulation restricted to the rostral fastigial nucleus in the cat can produce a marked elevation in blood pressure and an increase in the heart rate (Miura & Reis, 1969, 1970, 1971; Achari & Downman, 1969, 1970; Lisander & Martner, 1971). This response has been termed the fastigial pressor response (Miura & Reis, 1970). The nature of the associated changes in cardiovascular dynamics during the fastigial pressor response has only partially been characterized. Achari & Downman (1970) observed that the fastigial pressor response was associated with an adrenergically mediated decrease in the volume of the hind limb, kidney and intestine as well as pupillary dilatation, retraction of the nictitating membrane and an electrodermal response. These findings suggested that stimulation of the fastigial nucleus results in diffuse and widespread activation of the sympathetic nervous system. More recently Lisander & Martner (1971), estimating blood flow by measurement of the venous effluent, found that the response was differentiated, resembling in part the response to carotid occlusion. They did not however measure the cardiac output. The functional importance of the fastigial pressor response remains obscure.

If the fastigial pressor response consists of widespread sympathetic activation it would be unlike any pattern of cardiovascular response occurring to natural stimuli. However, further detailed analysis of the cardiodynamic changes characterizing the fastigial pressor response might provide a clue to its biological significance. In the present study, we have therefore attempted to define in detail by the use of relatively direct methods, the changes in regional blood flows, resistances, and pressures and the cardiodynamic events including heart rate, myocardial contractility and cardiac output characterizing the fastigial pressor response. We have found that the fastigial pressor response consists of a differentiated pattern of sympathetically mediated cardiovascular activity and that this pattern simulates that of the orthostatic cardiovascular reflexes elicited by maintenance of an upright posture. A preliminary report of this study has been published already (Doba & Reis, 1972a).

METHODS

A. General methods

Forty-five adult mongrel cats were anaesthetized with 1% α -chloralose (50 mg/kg i.v.) after induction with ether anaesthesia. After insertion of cannulae in the femoral or brachial artery, femoral vein, and trachea, the animal was placed in a rotating

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stereotaxic frame with the head flexed at 45 degrees. The rectal temperature was maintained at 37° C by a thermostatically regulated infrared lamp. Since the experiments usually lasted no more than 4–5 hr the initial dose of anaesthetic was considered sufficient to ensure surgical anaesthesia throughout the course of the experiment.

B. Measurement of cardiovascular activity

(i) *Systemic blood pressure* was recorded from a polyethylene catheter threaded up the femoral or brachial artery and positioned respectively in the abdominal aorta or the aortic arch and connected to Statham P23Db transducer. The heart rate was computed from the blood pressure pulse by a cardiometer (Beckman 9857) and end-tidal CO₂, sampled through a fine catheter placed in the tracheal cannula, was recorded by an infra-red gas analyser (Beckman LD-1). All cardiovascular activity was displayed on a polygraph (Beckman Dynograph Recorder, 504 A).

(ii) *Regional blood flow* was recorded by a square-wave electromagnetic flow meter (Carolina Medical Electronics, types 322 and 332). Flow probes (Carolina Medical Electronics, EP403R, EP404R) were applied to (a) the femoral artery just below the inguinal ligament (White & Ross, 1966); (b) the axillary artery at its junction with the aortic arch approached through a supraclavicular incision; (c) the renal arteries, approached retroperitoneally as described by Hoffer (1965) with care taken to avoid interruption of the renal nerves; (d) the superior mesenteric artery, approached through a mid line laparotomy with careful separation of the surrounding sympathetic nerve net to assure minimal damage to the nerves (Ross, 1967); (e) the common carotid artery, approached through a mid line incision in the neck with isolation of the artery from the connective tissue of the carotid sheath (Abel, Pierce & Guntheroth, 1963); (f) the external carotid artery, exposed by splitting sternocleidomastoid and retracting the digastric muscle; and (g) the ascending aorta, approached through a thoracotomy in which the sternum was split transversely at the level of the third intercostal space, the rib widely retracted, the pericardium incised, and the root of the aorta delivered for placement of the probe (Kumazawa, Baccelli, Guazzi, Mancina & Zanchetti, 1969). No more than two probes were inserted in the animal at any one time. However, in any one experiment flow changes in numerous beds might be sampled. Renal blood flow was never measured after a laparotomy since the operation may produce reflex effects on renal flow (Hoffer, 1965).

After placement of the flow probe, the appropriate incision was closed. Particular care was given to assure mechanical stability of the arterial probe. The zero level of the flowmeter was established *in situ* before and just after an experimental series by occluding the artery distally. At the termination of an experiment, the probes were calibrated by passing whole blood from a reservoir at several constant flow rates through the isolated arterial segment to which the probe was attached (Kumazawa *et al.* 1969). In all animals a ligature was tightly tied around the ankle or forepaw to eliminate blood flow to the foot pad. This assured that the femoral or axillary arterial flow reflected blood flow to muscle (Feigl & Folkow, 1963) and not skin. In some animals the limbs were skinned to further exclude cutaneous blood flow. In all instances mean blood flow was recorded through an integration circuit built into the flowmeter and having a time constant of 0.5 sec.

The mean arterial pressure (P_m) was derived from the formula $(P_s + 2P_d)/3$, where P_s was systolic pressure and P_d was diastolic pressure. Regional vascular resistance (RVR) was conventionally obtained from the formula; $RVR = P_m/F_m$, where F_m was mean blood flow in a given vascular bed.

(iii) *Cardiac output* was estimated either from the blood flow in the ascending aorta (Kumazawa *et al.* 1969) or by a thermal dilution technique (Fegler, 1954;

Korner, 1965; Folkow, Lisander, Tuttle & Wang, 1968). In the latter method a small thermistor (Model 14012A, Hewlett-Packard) was threaded down the common carotid artery to lodge at the aortic arch just above the aortic valve. The thermistor probe served as one arm of a Wheatstone bridge circuit with the remainder of the bridge having variable voltage and calibration resistance and contained in a coupler (Hewlett-Packard 350-15) mounted in the polygraph. The thermistor was a special glass-coated element with a resistance logarithmically inversely proportional to temperature changes. Nominal resistance of the probe at 40 °C was $1200 \Omega \pm 5\%$. The thermistor probe was pre-calibrated by the manufacturer to a corresponding absolute temperature for each ohm increment of thermistor resistance over a range of 1.000–2.999 k Ω (equivalent to a temperature range of 15–45° C) and matched to a corresponding balancing resistance. 0.9% saline (1 ml.) at room temperature (Evonuk, Imig, Greenfield & Eckstein, 1961) was injected as a bolus into the right atrium from a catheter threaded down the jugular vein and the thermal dilution curve recorded. In the cat there is a significant recirculation of thermal indicator as evidenced by a change in the slope of the down stroke of the curve. To eliminate recirculation from the calculation the down stroke of the curve was corrected by re-plotting the curve on semi-logarithmic paper and the area measure by a planimeter. Cardiac output was calculated according to the method described by Korner (1965).

Calculation of the cardiac output was made from the following formula:

$$C.O. = \frac{Q_i \times (T_b - T_i) \times K}{t \times T_a}$$

where C.O. = cardiac output in ml./min; Q_i = quantity of injectate in ml.; T_b = temperature of blood in degrees C; T_i = temperature of injectate in degrees; T_a = average temperature change; t = time in sec; $(t \times T_a)$ = area under curve; K = a constant;

$$K = \frac{\text{specific gravity of injectate} \times \text{specific heat of injectate} \times 60 \text{ (sec)}}{\text{specific gravity of blood} \times \text{specific heat of blood}}$$

$$K = \frac{1.005 \times 0.907}{1.054 \times 0.88} \times 60 = 64.82.$$

The values for specific gravity and specific heat were obtained from the Handbook of Biological Data (1952) and the formula of Korner (1965).

(iv) *Ventricular contractile force* was measured by a strain gauge arch (Cotten & Bay, 1950) (resistance 120 Ω) attached to the right ventricular wall by silk sutures. Details of the construction of this instrument have been reported elsewhere (Bonifacc, Brodie & Waldon, 1953). The distance between the two legs of the arch is variable and ranges between a minimum of 15 and maximum of 30 mm. To obtain the maximum measurable contraction the arm distance was set at approximately 150% of the initial length (Cotten & Bay, 1956). The strain gauge arch was calibrated in terms of the gram weight required to produce a given deflexion of the pen on the polygraph. The instrument was connected through a strain gauge coupler (Beckman 9803) to the polygraph.

(v) *Central venous pressure* was measured through a polyethylene catheter inserted into the right atrium through the jugular or femoral vein. To obtain the occluded vein pressure in the femoral vein a pressure cuff was applied to the upper part of the thigh and inflated to a level above systolic pressure, around 250 mm Hg (Browse, Lorenz & Shepherd, 1966). A polyethylene catheter was inserted into the

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vein distal to the site of occlusion and connected via a Statham P23Db transducer to the polygraph.

(vi) *Other manipulations.* The vagus nerve was sectioned in the neck. Transection of the cardiac nerves distal to the stellate ganglion was performed through a thoracotomy as described above. The renal nerves were transected through a retro-peritoneal approach and the femoral artery was denervated by section of both the sciatic and femoral nerves (White & Ross, 1966). Pupillary size and retraction of the nictitating membrane were estimated by visual observation and in some cases documented by photography.

C. Stimulation of the fastigial nucleus

The rostral and ventromedial quadrant of the fastigial nucleus, the site at which a fastigial pressor response is evoked (Miura & Reis, 1969), was stimulated electrically through a thin monopolar electrode consisting of a stainless-steel wire (diameter 0.006 in.) coated with teflon and bared at the tip for 0.3 mm and carried in a number 28 stainless-steel hypodermic needle. The electrode was inserted in the cerebellum through a small hole drilled with a dental drill through the occipital bone above the nuchal ridge. The anode was a clip attached to a scalp muscle. The fastigial nucleus was stimulated with a 12 sec train of square wave pulses of 0.1 msec. duration at a stimulus frequency of 50 Hz, delivered to the animal from a pulse generator (Devices, Digitimer) through a stimulus isolation unit (Devices, MK IV). The stimulus current was measured by passing the output from the stimulator through a 10 Ω resistor and the voltage drop across this resistor was amplified by a Tektronics type 122 preamplifier and displayed on a cathode ray oscilloscope (Tektronics 300) where it was continuously monitored throughout the experiment. The pressor response was established as not due to spread of the stimulus current to the brainstem by previously defined criteria (Miura & Reis, 1969). These were the absence of facial twitching, a frequency maximum for the response between 30 and 80 Hz, and evidence at the end of the experiment that a punctate lesion placed at the electrode site abolished the evoked response.

After placement of the stimulating electrode the animal was paralysed with gallamine-triethiodide (5 mg/kg) and artificially ventilated by a Harvard respirator pump maintaining end-expired CO_2 at 2-3%. The threshold response was arbitrarily defined as a rise of the mean blood pressure greater than 10% of control. After establishing the threshold stimulus intensity the fastigial nucleus was stimulated at intensities of 1-5 times threshold, a range of stimulus intensities which does not result in a spread of the stimulus to the brainstem (Miura & Reis, 1970). In the usual experiment, after placement of a stimulating electrode and paralysing the animal, base line measurements of specific cardiovascular parameters were taken. After the animal had stabilized, a series of observations was taken before, during and after stimulation of the fastigial nucleus. The significance of changes in cardiovascular function was estimated by a paired *t* test (Snedecor & Cochran, 1967). At the termination of the experiment the stimulus site was marked by passage of a 20 μA current for 30 sec in order to deposit iron at the electrode tip.

D. Histological confirmation

At the termination of the experiment the animal was perfused with 10% formaldehyde and 1% potassium ferro- and ferri-cyanide in order to identify the position of the tips of the stimulating electrodes by the Prussian blue reaction (Crill & Reis, 1968). The brain was then fixed frozen and sectioned at 50 μ . The sites of iron deposition were identified before and after staining the sections for either cells (Nissl) or fibres (Weil).

RESULTS

A. Changes in arterial blood pressure and regional blood flow during the fastigial pressor response

As previously described (Miura & Reis, 1969, 1970, 1971; Achari & Downman, 1970; Lisander & Martner, 1971) electrical stimulation of the ventromedial portion of the rostral fastigial nucleus (fastigial pressor area) (Fig. 1) resulted in an elevation of systolic, diastolic and mean blood pressures (Table 1, Fig. 2). The pressure responses were graded (Fig. 2) and highly reproducible from cat to cat. Stimulation at 5 times the threshold increased the systolic blood pressure to 189% of control.

TABLE 1. Changes in cardiovascular dynamics associated with electrical stimulation of fastigial nucleus. Fastigial stimulation delivered with stimulus currents $5 \times$ threshold. All values expressed as mean \pm s.e. of mean. n.s. = not significant

	N	Control	Fastigial stim.	Change (%)	P
<i>Systemic arterial pressure (mm Hg)</i>					
Systolic pressure	29	130 \pm 4	241 \pm 6	+89	< 0.001
Diastolic pressure	29	99 \pm 4	176 \pm 4	+83	< 0.001
Mean pressure	29	110 \pm 4	198 \pm 4	+82	< 0.001
Pulse pressure	29	29 \pm 2	64 \pm 3	+141	< 0.001
<i>Venous pressure (cm H₂O)</i>					
Central venous pressure	5	3.2 \pm 0.7	3.6 \pm 0.7	+13	n.s.
Occluded vein pressure	9	15.6 \pm 0.9	17.0 \pm 1.1	+8	n.s.
<i>Cardiodynamics</i>					
Heart rate (beat/min)	29	223 \pm 5	248 \pm 5	+12	< 0.01
Cardiac output (ml./min)	11	422 \pm 9	431 \pm 10	+2	n.s.
Stroke volume (ml./beat)	11	1.83 \pm 0.01	1.69 \pm 0.03	+8	< 0.05
Cardiac contractile force (g)	4	31.8 \pm 1.5	41.1 \pm 1.4	+30	< 0.01
<i>Regional blood flow (ml./min)</i>					
Femoral artery	29	11.7 \pm 0.4	9.0 \pm 0.5	-23	< 0.001
Axillary artery	1	10.7 \pm 0.7	8.7 \pm 1.0	-23	< 0.05
Renal artery	26	27.3 \pm 0.7	13.6 \pm 1.5	-48	< 0.001
Mesenteric artery	6	12.3 \pm 1.0	8.6 \pm 1.0	-26	< 0.05
Common carotid artery	9	17.8 \pm 1.3	30.7 \pm 2.5	+96	< 0.001
Ascending aorta	8	345 \pm 6	339 \pm 22	-1.6	n.s.
<i>Regional vascular resistance (mm Hg. ml⁻¹/min.)</i>					
Femoral artery	29	10.3 \pm 0.5	24.0 \pm 1.8	+132	< 0.001
Axillary artery	10	10.5 \pm 0.6	26.2 \pm 2.7	+159	< 0.001
Renal artery	26	3.7 \pm 0.2	14.7 \pm 2.3	+320	< 0.001
Mesenteric artery	6	9.5 \pm 0.4	22.0 \pm 2.8	+131	< 0.01
Common carotid artery	9	7.3 \pm 0.7	0.6 \pm 0.4	-6	n.s.
Ascending aorta	8	0.31 \pm 0.03	0.58 \pm 0.03	+91	< 0.01

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Associated with the increase in blood pressure is a decrease in blood flow to the femoral, renal, axillary and mesenteric arteries. The decrease in flow is graded (Figs. 2, 3) and reflects an increase in vascular resistance (Table 1, Fig. 3). With the exception of renal blood flow, the changes in blood flow were sustained during the 12 sec stimulus. In the renal artery

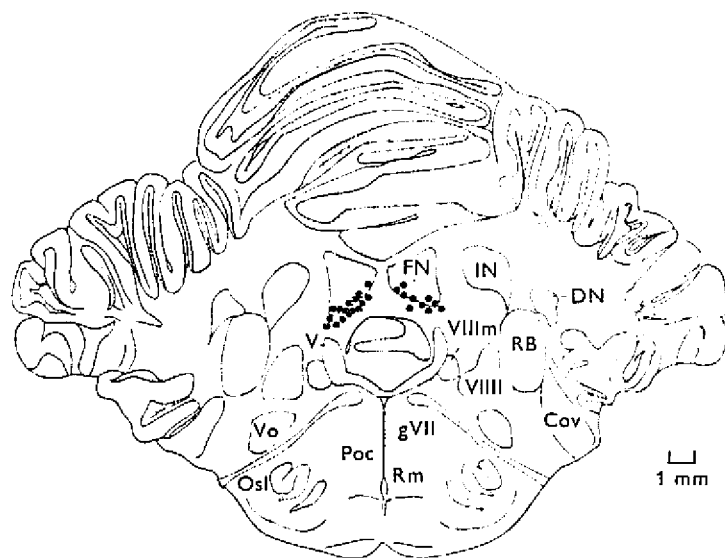


Fig. 1. Localization of sites in fastigial nucleus in twenty-six consecutive experiments from which a maximal pressor response was evoked in anesthetized cats. The positive sites (filled circles) are displayed on a representative coronal cross-section of the brain stem at approximately the level of the rostral quarter of the nucleus. Abbreviations, according to Taber (1961): Cov, nucleus cochlearis dorsalis; DN, nucleus dentatus; FN, nucleus fastigii; gVII, genus nervi facialis; IN, nucleus interpositus; Osl, nucleus olivaris superior lateralis; Poc, nucleus pontis centralis caudalis; RB, corpus restiformis; Rm, nucleus raphe magnus; V, IV ventricle; Vo, nucleus tractus spinalis oralis; VIII, nucleus vestibularis lateralis; VIIIIm, nucleus vestibularis medialis.

the blood flow tended to drift back to control values during stimulation possibly as a consequence of renal autoregulation (Johansson, Sparks & Biber, 1970). A rebound overshoot of blood flow and a slowing of the heart rate often occurred after termination of the stimulus (Fig. 2).

The relative magnitude of changes in flow and resistance in different arteries is tabulated in Table 1. The greatest change occurred in the renal artery with vascular resistance increasing fourfold in contrast to the doubling of resistance in the femoral, axillary and mesenteric arteries. This greater responsiveness of the renal artery is also reflected in a stimulus response curve which is steeper than that of other vessels (Fig. 4).

Blood flow in the ascending aorta did not change during the fastigial pressor response (Fig. 5, Table 1) which, as described below, is an indication of an unchanged cardiac output during the response. However, the increase in resistance in the ascending aorta to 191% of control indicates that the total peripheral resistance is almost doubled.

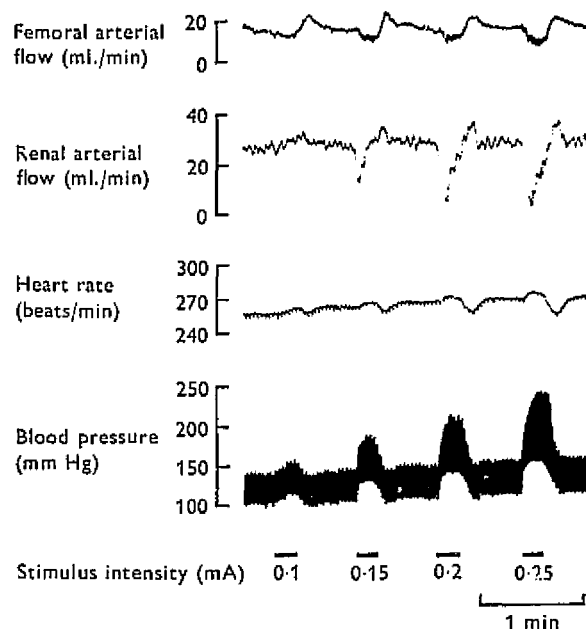


Fig. 2. Changes in femoral arterial blood flow (upper channel) renal arterial blood flow (second channel from top), heart rate (second channel from bottom) and aortic blood pressure (bottom channel) during graded electrical stimulation of the fastigial nucleus in a paralysed anaesthetized cat. The standard stimulus in this and subsequent illustrations consists of a 12-sec train of square-wave pulses of 0.1 msec duration delivered at 50 Hz (black bars at bottom of record). Stimulus intensity is enumerated in mA.

In several experiments blood flow was recorded simultaneously in both femoral or in both renal arteries. At no time was there any evidence of laterality of the response.

In contrast to the other vascular beds, blood flow in the common carotid artery increased during the fastigial pressor response (Table 1, Fig. 6). There are several lines of evidence suggesting that the increase in carotid blood flow reflects an increase in cerebral blood flow. First, the increased carotid flow persisted after removal of all muscles of the cranium, a large recipient of blood flow from the external carotid artery. Secondly, direct visualization of the pial vessels (exposed through a small hole placed in the calvarium and dura) through a dissecting microscope (at 12.5×

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magnification) during the fastigial pressor response revealed a marked increase in arteriolar and venular flows and reddening of the cerebral veins.

The increased carotid blood flow during the fastigial pressor response was unassociated with any change in carotid arterial resistance (Fig. 7) and was of equal magnitude, when expressed as percent of control, to the elevation of blood pressure (being 96 % and 89 %, respectively; Table 1).

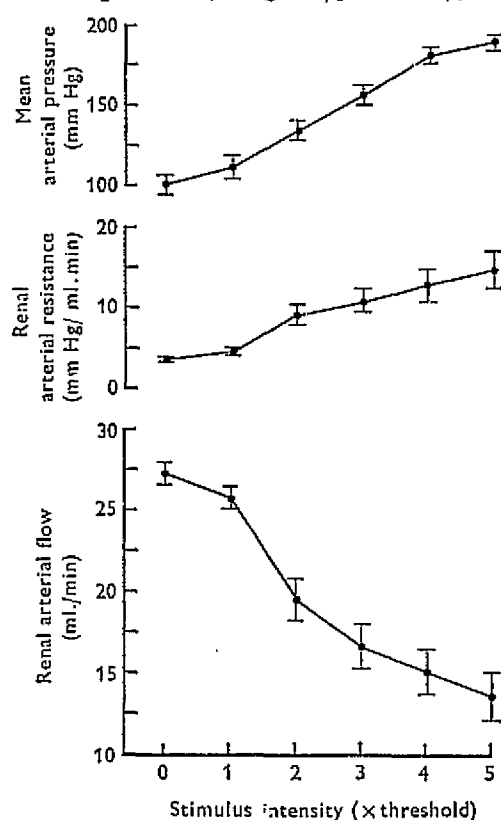


Fig. 3. Changes in mean arterial pressure (upper graph), renal arterial resistance (middle graph) and renal arterial blood flow (lower graph) during graded stimulation of the fastigial nucleus in anaesthetized paralysed cats. Each point represents mean \pm S.E. of mean ($n = 26$).

This indicates that the change in cerebral blood flow is passive. This passive increase in flow during the fastigial pressor response is not found during hypertension produced by infusion of angiotensin II or norepinephrine. With pressor agents carotid arterial resistance increases due to autoregulation (Lassen, 1959; Sokoloff, 1949). The absence of resistance changes during the fastigial pressor response therefore suggests that autoregulation of the blood flow is suspended.

The vasoconstriction in the femoral, mesenteric, renal and axillary arteries as well as the hypertension were abolished by the systemic injection of the α -adrenergic blocking agent phentolamine (1 mg/kg i.v.) in four cats. This dose of the drug, however, did not alter the heart rate.

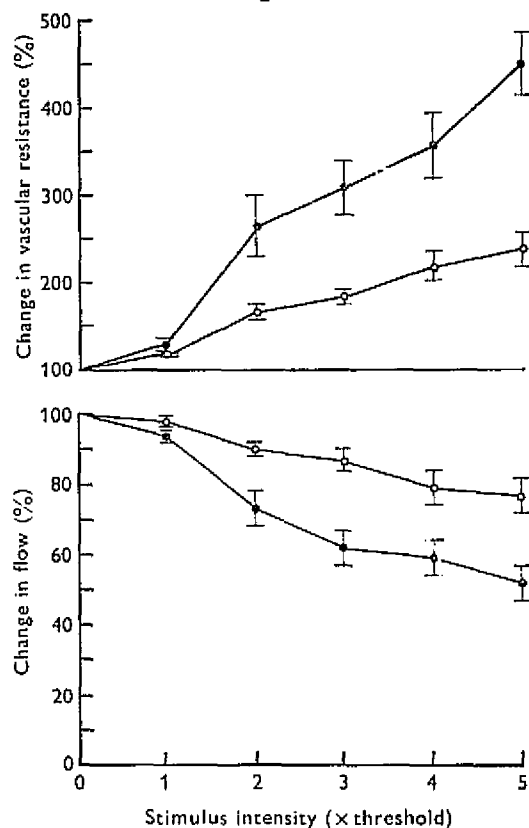


Fig. 4. Comparison of percentage change in arterial resistance (upper graph) and flow (lower graph) in renal (filled circles) and femoral (open circles) arteries during graded stimulation of the fastigial nucleus. Each point represents mean \pm s.e. of mean ($n = 26$ for the renal artery and 29 for the femoral artery). Note the steep stimulus/response curve for renal arterial flow and resistance during the fastigial pressor response.

Surgical interruption of the renal (Fig. 8), femoral or sciatic nerves also reversed the local changes of flow from a decrease to an increase. Bilateral adrenalectomy did not affect the magnitude of the blood pressure or flow changes during the fastigial pressor response. The increase in peripheral vascular resistance during the fastigial pressor response therefore results from activity of post-ganglionic sympathetic neurones and is mediated by α -adrenergic receptors.

B. Changes in venous pressure

During the fastigial pressor response there were no changes in the central venous pressure (Table 1), nor in pressure measured in a distal segment of the femoral vein occluded proximally (occluded vein pressure). This contrasts with the increase in central venous pressure associated with the defence reaction evoked by hypothalamic stimulation in cat (Lisander 1970).

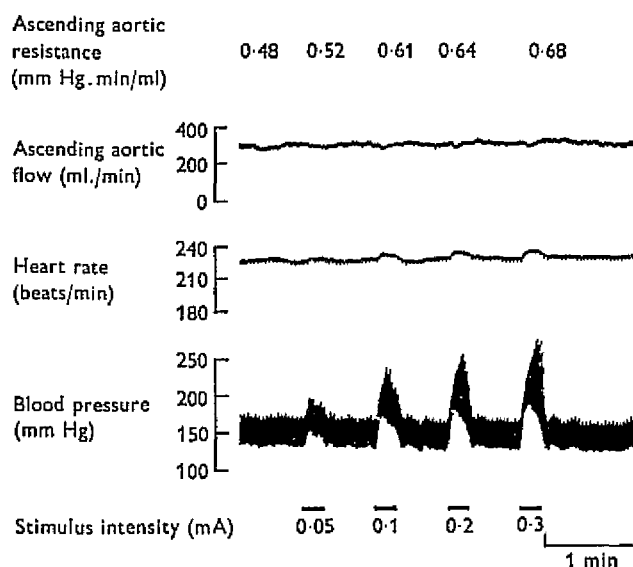


Fig. 5. Blood flow in ascending aorta (top channel), heart rate (middle channel), aortic blood pressure (bottom channel) and calculated resistance (numbers above aortic blood flow) during graded electrical stimulation of the fastigial pressor area in an anaesthetized paralysed cat. Note increase in resistance without significant change in aortic flow during fastigial stimulation.

C. Changes in cardiac function during the fastigial pressor response

During the fastigial pressor response there is a small but significant increase in the heart rate (Table 1, Figs. 2, 5, 8) sometimes associated with ventricular ectopic beats, and also an increase in myocardial contractile force (Fig. 9). Both changes in heart rate and contractility were abolished by transection of the cardiac nerves at the stellate ganglion (Fig. 9) or by intravenous injection of the β -adrenergic blocking drug propranolol (1 mg/kg i.v.). Thus the changes in heart rate and force evoked by stimulation of the fastigial nucleus were neurogenic and mediated by cardiac β -receptors.

Despite the increase in heart rate and myocardial contractile force the cardiac output was unchanged during the fastigial pressor response whether measured by ascending aortic flow (Fig. 5) or by the thermal dilution technique (Table 1). The unchanged cardiac output therefore must have resulted from a decrease in stroke volume (Table 1). The reduced stroke volume was presumably in turn the result of an increased afterload secondary to the augmented peripheral resistance (Kirchheim & Gross, 1971).

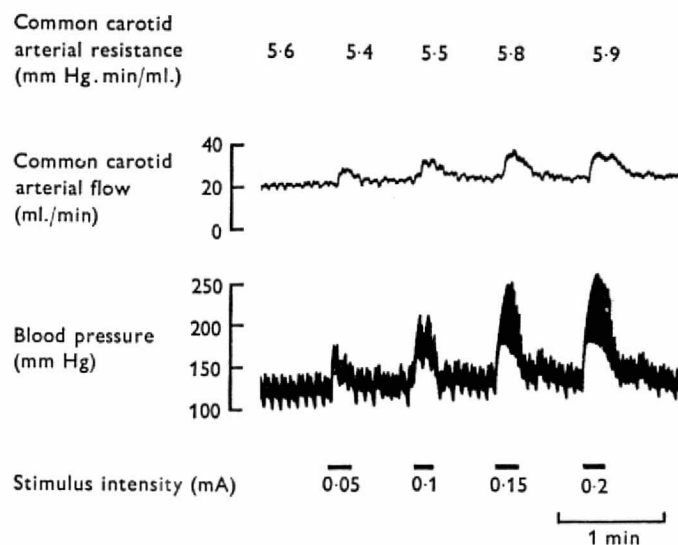


Fig. 6. Blood flow in common carotid artery (upper channel) aortic blood pressure (lower channel) and calculated resistance (above carotid flow channel) in the common carotid artery during graded stimulation of the fastigial pressor area in an anaesthetized paralysed cat. Note increase in flow without change in resistance during the fastigial pressor response.

D. Pupillary changes

There was minimal (less than 1.5 mm) dilatation or no change in pupillary diameter and no retraction of the nictitating membrane during the fastigial pressor response when elicited by electrical stimulation of $5 \times$ threshold (Fig. 10). The absence of pupillary signs during the fastigial pressor response was not the result of inexcitability of the central peripheral pupillomotor pathways since stimulation of the postero-lateral hypothalamus in several experiments evoked, along with an elevation of blood pressure, a brisk bilateral retraction of the nictitating membrane and bilateral pupillary dilatation to 70–80% of the maximum dilatation effected by stimulation of the distal end of the sympathetic nerve in the neck (Fig. 10).

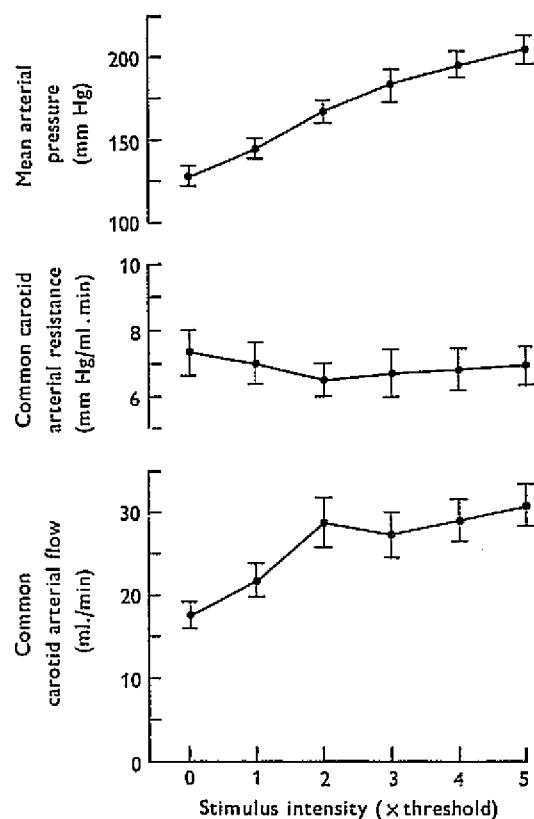


Fig. 7. Changes in mean arterial pressure (upper graph), common carotid arterial resistance (middle graph) and common carotid arterial blood flow (lower graph) during graded electrical stimulation of the fastigial pressor area in anaesthetized paralysed cats. Each point represents mean \pm S.E. of mean ($n = 9$).

DISCUSSION

The present study indicates that stimulation of the rostral fastigial nucleus produces a complex, stereotyped and graded pattern of cardiovascular activity mediated by the sympathetic nervous system. The preponderant response to such stimulation is an alpha-adrenergically mediated constriction of the arteries to skeletal muscle (as evidenced by decreased flow in the femoral and axillary arteries), to kidney and to intestine. These findings are qualitatively in accord with the observations of Achari & Downman (1970) and Lisander & Martner (1971) who estimated blood flow by plethysmographic or drop recorder techniques respectively. As a consequence of the vasoconstriction there is a marked increase in the

peripheral vascular resistance resulting in an elevation of the systemic blood pressure.

Despite a β -adrenergically mediated increase in heart rate and myocardial contractility there is no change in the cardiac output. This is probably the consequence of the increased left ventricular afterload resulting from the increase in peripheral resistance thereby reducing the left ventricular stroke volume. This effect is similar to the mechanism responsible for the reduction in cardiac output induced by injection of vaso-active substances or by acute experimental hypertension (Olmsted & Page, 1965*a, b*).

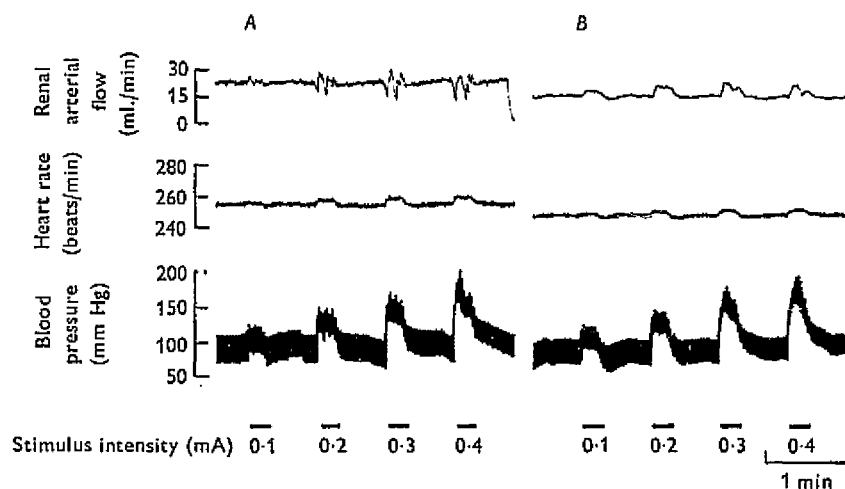
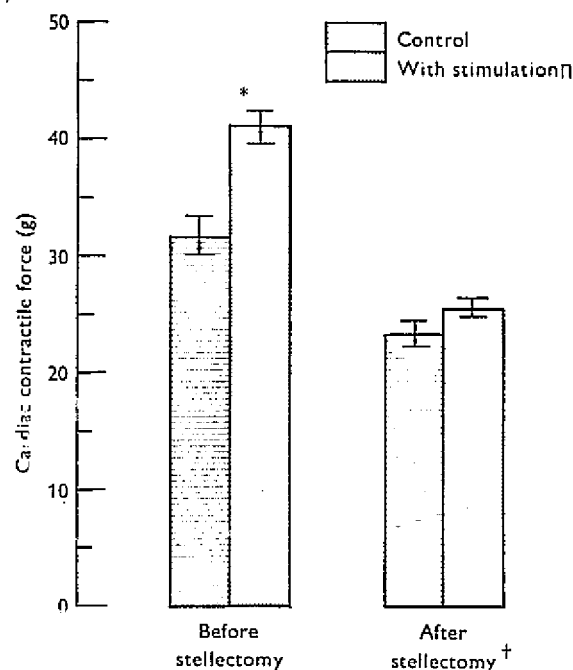


Fig. 8. Effects of transection of renal nerves on renal blood flow during the fastigial pressor response. Upper channel: renal arterial flow. Middle channel: heart rate. Lower channel: aortic blood pressure. *A*, before nerve transection. *B*, after nerve transection. Note reversal of blood flow from a reduction to an increase after nerve transection.

In addition to the direct activation of the sympathetic cardiovascular neurones there is simultaneously an inhibition of both the cardiovagal and the vasomotor components of the baroreceptor reflexes (Achari & Downman, 1970; Miura & Reis, 1971; Lisander & Martner, 1971). Such inhibition might serve, by blocking the bradycardia, to maintain the cardiac output in the face of the increased peripheral vasoconstriction.

The pattern of activation of the sympathetic neurons during the fastigial pressor response is differentiated, i.e. results from a selective rather than diffuse activation of sympathetic neurons. Thus, despite intense activation of sympathetic vasoconstrictor neurones supplying skeletal muscle, kidney and gut there is no sign of increased venoconstriction as reflected in the

central venous pressure or occluded vein pressure nor any change in the common carotid arterial resistance. Nor is there pupillary dilatation or retraction of the nictitating membrane. These observations are in accord with recent evidence demonstrating that the sympathetic nervous system has a highly developed capacity to discharge selectively in an organ and function specific manner (e.g. Feigl, 1964; Hoffer, 1965; Clark, Smith & Shearn, 1968).



*Differs from control $P < 0.05$

†Differs from unoperated control $P < 0.01$

Fig. 9. Effects of stellate ganglionectomy on the increase in cardiac contractile force associated with the fastigial pressor response. Note that the increase in contractile force during fastigial stimulation just reaches threshold) is abolished by stellatectomy. Stellate ablation also significantly reduces pre-stimulation myocardial contractility.

Although all the component parts characterizing the fastigial pressor response have been elicited alone or in partial combinations by brain stimulation (Feigl, 1964; Ueda, Inoue, Iizuka, Izuka Ithori & Yasuda, 1966), fastigial pressor response in its totality represents a patterned and stereotyped cardiovascular response unlike any heretofore evoked by electrical stimulation in brain or periphery. Its reproducibility and stereotypy are also distinctive. For example, it is unlike the defence reaction (Abrahams,

Hilton & Zbrozyna, 1960; Folkow *et al.* 1968; Lisander, 1970) or exercise responses (Smith, Rushmer & Lasher, 1960) in which an elevated blood pressure and tachycardia are associated with increased flow to muscle and an increase in the cardiac output. It also differs from peripherally or centrally induced reflex responses to hypoxia (Downing, Remensnyder & Mitchell, 1962; Daly & Scott, 1963; Downing, Mitchell & Wallace, 1963;

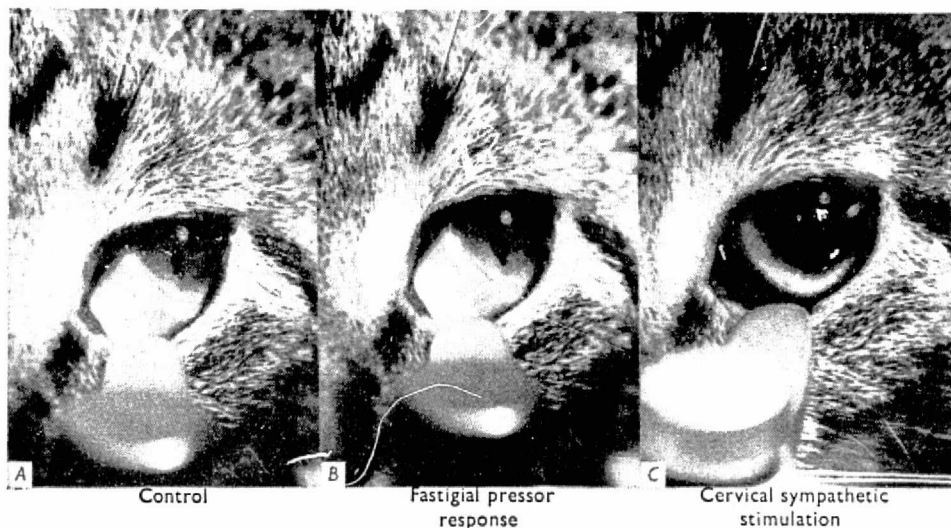


Fig. 10. Absence of pupillary dilatation or retraction of nictitating membrane during the fastigial pressor response. *A*, control. *B*, during the fastigial pressor response at 5 times threshold. *C*, electrical stimulation of vago-sympathetic nerve. The cut distal end of the nerve was placed on a bipolar electrode and stimulated with an electrical stimulus pulse train of square wave pulses of 0.1 msec duration delivered at 50 Hz supramaximal for pupillary dilatation.

Daly, Hazzledine & Howe, 1965; Reis & McHugh, 1968) and diving responses (Feigl & Folkow, 1963; Yonce & Folkow, 1970) in which vasoconstriction in limb and visceral beds is associated with a bradycardia. However, comparison with other cardiovascular responses in which vasoconstriction and tachycardia are prominent reveals that the fastigial pressor response closely resembles the integrated cardiovascular response to maintenance of an upright posture (the orthostatic reflexes). It also, in large measure, simulates the differentiated response to carotid sinus hypotension or occlusion (Lisander & Martner, 1971) which is believed to be the principal stimulus to the orthostatic reflexes (Gauer & Thron, 1965).

We would therefore propose that the cardiovascular responses associated with the fastigial pressor response result from excitation of the neural networks which subserve the orthostatic reflexes. Further support for this

contention is our finding that small lesions within the bilateral fastigial pressor areas but not elsewhere in the cerebellum will impair the compensatory cardiovascular responses to headup tilting in the paralysed anaesthetized cat (Doba & Reis, 1972*a, b*).

That the fastigial nucleus is involved in postural cardiovascular reflexes is not surprising in view of its critical participation in somatic postural responses. Moreover the rostral fastigial nucleus receives a projection from the anterior mid line vermis (Jansen & Brodal, 1940) the only portion of the cerebellar vermis from which cardiovascular changes have been consistently elicited by electrical stimulation (Hoffer, 1965). Stimulation of the anterior vermis produces, in general, increased flows to skeletal muscle due to sympathetic inhibition often associated with decreased renal flow, a pattern more appropriate to the defence reaction or exercise than to postural responses. This raises the interesting possibility that the Purkinje cells of the anteromedial cerebellar cortex may produce phasic increases in muscle flow in relationship to movement by inhibiting neurons of the fastigial nucleus (Eccles, Ito & Szentogothai, 1967; Ito, Yoshida, Obata, Kawai & Udo, 1970). The role of the fastigial nucleus in autonomic postural reflexes postulated here would serve as another demonstration of the intimate functional coupling between the somatic and autonomic motor system in the brain.

This study was supported by research grants from the National Institutes of Health (NS 04876), from the National Aeronautics and Space Administration (NGR 33-010-179) and the Harris Foundation. Elsa Orejuela and Nancy Shieh provided excellent technical support.

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**Acute Fulminating Neurogenic Hypertension
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ABSTRACT

Bilateral electrolytic lesions of the nucleus tractus solitarius in the rat at the level of the obex abolished baroreceptor reflexes and resulted in an immediate, marked elevation in systemic blood pressure without a change in heart rate. In unanesthetized rats the hypertension was associated with a marked increase in total peripheral resistance, a reduction in blood flow in the abdominal aorta, and an increase in central venous pressure. The cardiac output was reduced to 62% of control as a consequence of reduced stroke volume, which was reflected, in turn, by increased end-diastolic pressure. The hypertension was abolished and the end-diastolic pressure lowered by blockade of alpha receptors with phentolamine. The hypertension was not due to changes in blood gases or to release of agents from the kidneys or the adrenal glands; it was very sensitive to anesthetics and was abolished or aborted by midcollicular decerebration. Within hours after lesioning, the rats developed progressive congestive heart failure and died in shock, often in association with pulmonary edema. We concluded that the fulminating hypertension evoked by lesions of the nucleus tractus solitarius was due to the increased vasoconstriction caused by the augmented discharge of sympathetic nerves in response to central deafferentation of baroreceptor reflexes; the hypertension was mediated by alpha receptors and depended on the integrity of structures lying above the midbrain.

KEY WORDS

systemic vasoconstriction
vasomotor centers

baroreceptors

cardiac failure

nucleus tractus solitarius
pulmonary edema

■ Denervation of the carotid sinus and the aortic depressor nerves in several mammalian species results in a sustained elevation of systemic arterial blood pressure (1-9). This form of hypertension is neurogenic: it is due to an increased discharge of spinal preganglionic neurons resulting from the removal of an inhibitory drive to brainstem neurons from systemic baroreceptors (10).

Lesions of regions of the brainstem in which the baroreceptors terminate, i.e., the area of the nucleus tractus solitarius (NTS) at the level of the obex or the paramedian reticular nucleus (11-16), should also cause the development of hypertension. Although bilateral lesions of the NTS or of the paramedian reticular nucleus in a cat, which has been anesthetized or decerebrated at the midcollicular level, either abolish or attenuate baroreceptor reflexes, they do not result in hypertension (17).

The failure of such lesions to produce hypertension might be due to the fact that lesions destroy neurons mediating both the pressor and the depressor components of baroreceptor and chemoreceptor reflexes. Also, anesthesia or decerebration might mask any hypertensive response resulting from the lesions.

In this study, we attempted to produce hypertension in chronically prepared unanesthetized rats by bilateral electrolytic lesions of the NTS. We demonstrated that acute fulminating hypertension, often culminating in pulmonary edema, could be produced by such lesions.

Methods

These experiments were performed on male Sprague-Dawley rats (300-400 g) housed four to six in a cage in a thermostatically regulated room (20°C) with cycled lighting (on at 7 AM, off at 7 PM). They were provided with lab chow and water ad libitum. All rats were anesthetized with 2% halothane in 100% O₂ blown over the nose through a face mask. In most rats, a polyethylene catheter (PE50, 0.023 inches, i.d.) filled with heparinized saline (20 units/ml) was inserted in the ventral artery of the tail or the femoral artery for direct measurement of intra-arterial blood pressure. The catheter was fixed to soft tissue with sutures and connected to a strain-gauge transducer (Statham

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This work was supported by U. S. Public Health Service Grant NS 08476 from the National Institutes of Health, by Grant NGR 33-010-179 from the National Aeronautics and Space Administration, and by the Harris Foundation.

Received December 11, 1972. Accepted for publication March 12, 1973.

P23Db). Pressure measurements were displayed on a polygraph (Beckman Dynograph recorder 504A). Heart rate was computed from the blood pressure pulse wave by a cardiometer (Beckman 9857) and was simultaneously displayed. At this time, in selected rats, cannulas or probes for measuring cardiac output, aortic blood flow, left ventricular pressure, and central venous pressure were inserted. In most cases the anesthesia was then discontinued, and the rat was permitted to recover for 30 minutes. Base-line measurements of arterial blood pressure and heart rate were obtained in the quiet, awake state. The rat was then reanesthetized to place the brainstem lesions.

PRODUCTION OF HYPERTENSION BY LESIONS OF THE NUCLEUS TRACTUS SOLITARI

The rat was placed in a stereotaxic frame with the head flexed to 45°. The region of the obex was exposed by a limited occipital craniotomy. In some rats, a small portion of the posterior vermis of the cerebellum was removed by suction to facilitate exposure. A thin

$$K = \frac{\text{specific gravity of injectate} \times \text{specific heat of injectate} \times 60 \text{ (seconds)}}{\text{specific gravity of blood} \times \text{specific heat of blood}}$$

monopolar electrode consisting of a stainless steel wire (diameter 0.006 inches) coated with Teflon, bared at the tip (0.2 mm), and carried in a stainless steel hypodermic needle (no. 28) was placed in the brainstem by a micromanipulator. The lesion was made by passing an anodal d-c current of 5 ma for 1-3 seconds. The cathode was a clip placed in an adjacent muscle. The electrode was removed, and a lesion was then placed at a symmetrical site on the other side of the brainstem.

Several types of operated control rats were prepared. Sham operations were performed by exposing the brainstem and placing the electrode in the region of the NTS bilaterally without making lesions. In other controls subjected to surgery, bilateral lesions were placed in regions other than the NTS, usually laterally in the cuneate nucleus, ventrally in the medial reticular formation (sometimes including the raphe nuclei), or in the area postrema.

After surgery the wounds were closed and infiltrated with 2% procaine to minimize pain, and the rat was removed from the stereotaxic frame for further observation. When cardiovascular events were monitored, the rat was placed in a small cage through which cannulas or probes were led to appropriate connectors. Cardiovascular activity was measured within 30 minutes after cessation of anesthesia. At this time the rats were quiet, cardiovascular activity was reasonably stable, and, in rats with NTS lesions, the hypertension was well developed.

MEASUREMENT OF CARDIOVASCULAR ACTIVITY

Cardiac output was measured by a thermal dilution technique (7, 18, 19). A small thermister (Hewlett-Packard model 14012) was threaded down the common carotid artery and was lodged at the aortic arch just above the aortic valve. Normal saline (0.1 ml) at room temperature was injected as a bolus into

the right atrium from a polyethylene catheter of known fluid capacity threaded up the femoral vein (20). The thermal dilution curve was displayed on the polygraph. A significant recirculation of the thermal indicator occurred as evidenced by a change in the slope of the downstroke of the temperature curve (18). To eliminate any contribution of recirculation to the calculation of cardiac output, the curve was replotted on semilogarithmic paper, and the area under the curve was measured by a planimeter (7, 18). Cardiac output was then calculated according to the method of Cooper et al. (18):

$$CO = \frac{Q_i(T_b - T_i)K}{tT_a} \quad (1)$$

where CO = cardiac output (ml/min), Q_i = quantity of injectate (ml), T_b = temperature of blood (°C), T_i = temperature of injectate (°C), T_a = average temperature change, t = time (seconds), (tT_a) = area under curve, and K = a constant.

$$= \frac{1.005 \times 0.997}{1.054 \times 0.917} = 63.62.$$

The values used for specific gravity and specific heat of rat blood were those cited by Cooper et al (18).

Stroke volume was calculated by dividing cardiac output by heart rate.

Abdominal aortic blood flow was recorded by a square-wave electromagnetic flow meter. Through a laparotomy a flow probe 3 mm in circumference was applied to the abdominal aorta just below the origin of the renal arteries, and the incision was closed. The zero level of the flowmeter was determined by established methods and reconfirmed in situ at the end of the experiment after the rat was dead. The flow probe was precalibrated by passing whole blood through the probe at a constant rate (21, 22).

Total peripheral resistance (TPR) was calculated from the formula

$$TPR = (P_m - CVP) / CO, \quad (2)$$

where CVP = central venous pressure measured from the right atrium and P_m = mean arterial blood pressure.

$$P_m = (P_s + 2P_d) / 3, \quad (3)$$

where P_s = systolic pressure and P_d = diastolic pressure. Regional vascular resistance (RVR) was calculated from the formula

$$RVR = P_{a,i} / F_m, \quad (4)$$

where F_m = mean blood flow in the abdominal aorta.

Left ventricular pressure was measured through a polyethylene catheter (PE50, 0.023 inches, i.d.) threaded down the right common carotid artery into the left ventricle and connected to a Statham P23Db transducer.

Central venous pressure was measured through a polyethylene catheter threaded up the femoral vein into the right atrium.

BLOOD GAS ANALYSIS

PO_2 and PCO_2 were measured in 100- μ l samples of arterial blood collected in heparinized capillary tubes (Radiometer type D5511/12.5, 100 μ l) from the catheter placed in the ventral tail artery. The PO_2 and PCO_2 were measured in duplicate in a Radiometer Blood Microsystem (type BMS3) (23).

OTHER PROCEDURES

Decerebration was performed at the midcollicular level in anesthetized rats with a small blunt spatula inserted through a burr hole placed in the parietal bone. The cerebellum was removed by suction through a limited occipital craniotomy. The kidneys, the adrenal glands, or both were removed through a lateral flank incision. After placing ligatures around the renal hilus, the organs were removed en bloc without decapsulation of the kidney.

To test the baroreceptor reflexes, norepinephrine or angiotensin II, in a volume never exceeding 0.2 ml, was injected intravenously into a catheter in the femoral vein. The dose was sufficient to evoke a submaximal pressor response. These procedures were performed with the rat lightly anesthetized with alpha-chloralose (30 mg/kg, iv) to obtain a more stable blood pressure without impairing cardiovascular reflexes.

POSTMORTEM EXAMINATION

An autopsy was performed in rats that died spontaneously or were killed by an overdose of sodium pentobarbital (20 mg, ip). After ligation of the inferior and superior caval veins, ascending aorta, and trachea, the lungs and the heart were removed from the body and weighed. The lung weight-body weight ratio ($\times 100$) was used to assess the presence of pulmonary edema (24).

HISTOLOGICAL EXAMINATION OF BRAIN AND OTHER ORGANS

At the termination of the experiment the brain was excised and, along with other organs, placed in 10% formalin for at least 2 weeks. The localization of brain lesions was confirmed on frozen sections cut every 50 μ and stained for cells by the Nissl method (14). The lung and the heart were blocked, embedded in paraffin, and stained with hematoxyline and eosin.

STATISTICAL EVALUATION

The significance of changes in cardiovascular and other parameters resulting from brain lesions was estimated by a paired *t*-test (25) in which postlesion and prelesion measurements were compared. $P < 0.05$ was significant.

Results

Effects of Acute Lesions of the Nucleus Tractus Solitarius on the Blood Pressure, Heart Rate, Respiratory Rate, and Other Autonomic Responses.—Small bilateral electrolytic lesions of the dorsal brainstem that destroyed the NTS at the level of the obex invariably resulted in arterial hypertension (Fig. 1). The hypertension appeared within 5 minutes after the halothane anesthesia was

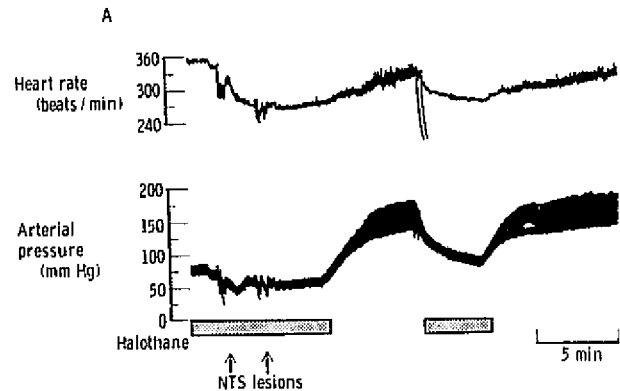


FIGURE 1

Typical blood pressure and heart rate responses elicited by bilateral lesions of the nucleus tractus solitarius (NTS) in the rat. At the arrows electrolytic lesions were placed in the NTS first on the left side and then on the right. The blood pressure rose to hypertensive levels immediately after discontinuation of halothane anesthesia and fell when the rat was reanesthetized. Arrhythmias were occasionally associated with the hypertension as is indicated by the downward deflections in the heart rate tracing.

stopped, and within 20–30 minutes hypertension was stable and sustained until the onset of heart failure. By 30 minutes after termination of anesthesia, the systolic, diastolic, and pulse pressures were significantly increased and unassociated with changes in heart rate (Table 1 and Fig. 1). The respiratory rate was generally reduced at this time to 85% of control (Table 1). The rats with NTS lesions were hypoactive, normothermic, and had no signs of any generalized increase in sympathetic activity such as proptosis, mydriasis, or piloerection.

Localization of Effective Lesions.—In all instances the electrolytic lesions of the brainstem effective in producing hypertension destroyed the bulk of the NTS and the adjacent solitary tract bilaterally at the level of the obex (Fig. 2 and Fig. 3a and b). The adjacent parahypoglossal area (26) and the dorsal motor nucleus of the vagus were variably damaged. Lesions primarily damaging the parahypoglossal areas with little damage to the NTS did not produce the syndrome. Partial lesions of the NTS, which often spared more lateral portions of the nucleus and the tract, were not sufficient to produce hypertension (Fig. 3c). Therefore, a critical mass of the NTS, or perhaps a specific portion of the nucleus and tract, apparently had to be destroyed bilaterally to produce hypertension. Moreover, damage to the parahypoglossal area was not, by itself, sufficient to produce hypertension. Unilateral NTS lesions resulted in a

TABLE 1

Effects of Bilateral Lesions of the Nucleus Tractus Solitarius in Rats on Cardiovascular Dynamics, Respiration, and Blood Gases

	N	Prelesion	Postlesion		P
			% of control		
Systolic pressure (mm Hg)	16	125 ± 3	201 ± 5	161	< 0.001
Diastolic pressure (mm Hg)	16	97 ± 2	151 ± 3	156	< 0.001
Pulse pressure (mm Hg)	16	28 ± 2	50 ± 4	179	< 0.001
Mean pressure (mm Hg)	16	106 ± 2	168 ± 3	158	< 0.001
Heart rate (beats/min)	16	411 ± 9	430 ± 13	105	ns
Cardiac output (ml/min)	12	119 ± 4	74 ± 3	62	< 0.001
Stroke volume (ml/beat)	12	0.29 ± 0.01	0.17 ± 0.01	59	< 0.001
Central venous pressure (cm H ₂ O)	12	1.8 ± 0.1	4.4 ± 0.1	244	< 0.001
Total peripheral resistance (mm Hg min/ml)	12	0.928 ± 0.001	2.376 ± 0.126	255	< 0.001
Left ventricular end-diastolic pressure (mm Hg)	6	15 ± 2	49.5 ± 5	326	< 0.001
Abdominal aortic flow (ml/min)	6	11.7 ± 0.6	7.3 ± 1.1	62	< 0.01
Abdominal aortic resistance (mm Hg min/ml)	6	7.9 ± 0.1	23.8 ± 4.1	301	< 0.001
Respiratory rate (breath/min)	20	64 ± 2	53 ± 3	85	< 0.01
Arterial PO ₂ (mm Hg)	7	93.1 ± 2.0	92.1 ± 1.4	99	ns
Arterial PCO ₂ (mm Hg)	7	39.6 ± 1.4	42.6 ± 1.6	107	ns

All values are means ± SE. Measurements in control rats and in rats with NTS lesions were taken 30 minutes after cessation of anesthesia. Statistical evaluation was made using a paired *t*-test (25) relating postlesion values to control values. NS = not significant.

small but significant elevation of blood pressure above control. However, these rats survived over 24 hours, at which time blood pressure measured directly had returned to normal.

Lesions in adjacent areas of the medulla including the cuneate nuclei (Fig. 3d), the raphé nucleus (Fig. 3e), and paramedial sites of the reticular formation (part of the so-called depressor zone [27]) (Fig. 3e) did not produce hypertension. Lesions of the area postrema also did not affect blood pressure.

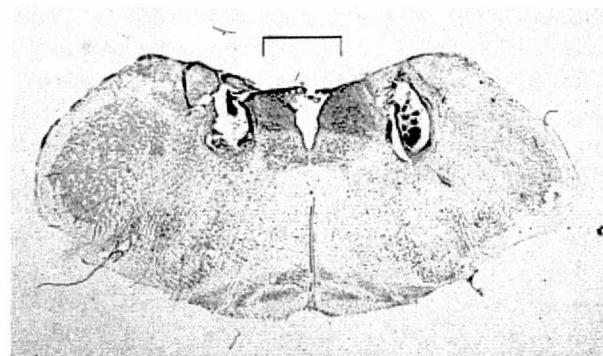


FIGURE 2

Representative lesion of the brainstem in a rat that produced fulminating neurogenic hypertension. This section was taken just rostral to the obex. Nissl stain; bar represents 1 mm. See Figure 3 for identification of the nuclear groups.

Cardiodynamic Changes during the Acute Hypertensive Stage.—We next sought to establish whether the acute hypertension produced by NTS lesions was due to an increase in total peripheral resistance or to an increase in cardiac output. Since arterial hypertension was fully developed in the lesioned rat immediately after halothane was stopped (Fig. 1), cardiovascular activity was measured within the first 30 minutes after discontinuing the anesthesia when the rat was hypomotile but well established in arterial hypertension.

The cardiodynamic changes associated with the hypertension induced by bilateral lesions of the NTS are listed in Table 1. The elevated arterial blood pressure was accompanied by an increase in the total peripheral resistance to 255% of control and a tripling of the resistance in the abdominal aorta. The increased aortic resistance, in large measure, reflected the increased resistance in arteries to muscle and skin of the trunk and lower extremities. Aortic blood flow was reduced by 38%. In contrast to the increase in total peripheral resistance, the cardiac output was reduced to 62% of control (Table 1, Fig. 4). There was a 326% increase in left ventricular end-diastolic pressure, indicating decreased ventricular ejection (Table 1). The central

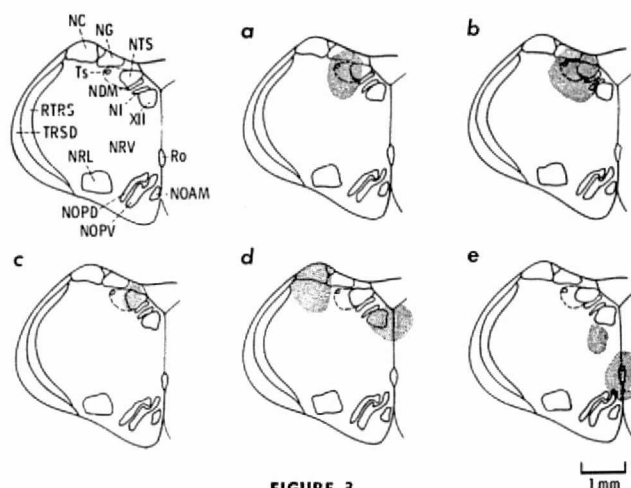


FIGURE 3

Representative brainstem lesions effective or ineffective in producing neurogenic hypertension in the rat. The lesions are projected on a cross section of the medulla of the rat at the level of the rostral third of the inferior olivary nucleus. Only one side of generally symmetrical lesions is shown. Lesions in a and b were associated with hypertension. The lesions in c, d, and e failed to produce hypertension. Abbreviations are according to Cragie (48): NC = nucleus cuneatus, NDM = dorsal motor nucleus of the vagus, NG = nucleus gracilis, NI = nucleus intercalatus, NOAM = nucleus olivaris accessorius, NOPD = nucleus olivaris principalis (pars dorsalis), NOPV = nucleus olivaris principalis (pars ventralis), NRL = nucleus reticularis lateralis, NRV = subnucleus reticularis ventralis medullae oblongatae, NTS = nucleus tractus solitarius, Ro = nucleus raphé obscurus, RTRS = radix tractus spinalis nucleus trigemini, TRSD = tractus spinocerebellaris dorsalis, Ts = tractus solitarius, XII = nucleus hypoglossi.

venous pressure measured in the right atrium was increased 244% (Table 1).

The arterial hypertension with the increased left ventricular end-diastolic pressure elicited by NTS lesions was promptly abolished by the systemic injection of the alpha-receptor blocking agent, phentolamine (1 mg/kg, iv). This finding indicates that the increased left ventricular end-diastolic pressure characterizing the acute hypertensive state resulted from an increased afterload that was, in turn, a consequence of the neurogenically mediated increase in peripheral vascular resistance.

Therefore, the hypertension produced by bilateral lesions of the NTS was primarily due to increased peripheral resistance, was neurogenic in origin, and was primarily mediated by alpha receptors.

Changes in Blood Gases during Acute Hypertension.—Because NTS lesions abolish chemoreceptor reflexes from carotid and aortic body chemoreceptors (17) and because the associated decrease in

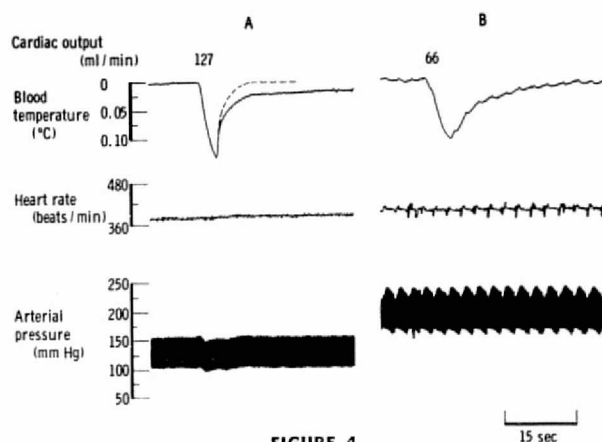


FIGURE 4

Changes in cardiac output, thermal dilution curves, heart rate, and systemic blood pressure before (A) and after (B) production of bilateral lesions of the NTS causing hypertension in the rat. During measurements the rat was unanesthetized. Before lesions were made, there was a significant recirculation apparent when the thermal dilution curve was replotted on a semilogarithmic paper (see Methods). The dotted line in A represents the curve corrected for recirculation. After bilateral lesions were made, there was practically no recirculation. The increased drop in temperature indicates that cardiac output decreased.

the respiratory rate could lead to a degree of asphyxia resulting in reflex hypertension (28, 29), arterial PO_2 and PCO_2 were measured in lesioned rats during the acute hypertensive phase. At the time the hypertension was well developed, the PO_2 and PCO_2 of arterial blood samples were unchanged. Moreover, administration of 100% O_2 by a face mask did not result in attenuation of the hypertension. Thus the hypertension resulting from lesions of the NTS was not due to a reflex response to hypoxia.

Effect of Removal of Adrenal Glands and Kidneys.—The hypertension produced by NTS lesions cannot be attributed to release of pressor substances from the kidneys or the adrenal glands. The magnitude of the hypertension was unaffected by prior bilateral removal of the adrenal glands and the kidneys (Table 2).

Effects of Anesthesia on Acute Hypertension.—The hypertension produced by NTS lesions was extremely sensitive to anesthetic agents. It disappeared if the rats were reanesthetized with 2% halothane (Fig. 1), barbiturates (e.g., sodium pentobarbital 40 mg/kg, iv), or alpha-chloralose (50 mg/kg, iv).

Effects of Midcollicular Decerebration and Cerebellectomy.—Decerebration at the midcollicular level either abolished the hypertension produced by

TABLE 2

Effects of Decerebration, Cerebellectomy, and Adrenonephrectomy on the Mean Blood Pressure in Rats with Bilateral Lesions of the Nucleus Tractus Solitarius

Operation	N	Control (mm Hg)	After NTS lesions (mm Hg)	After NTS lesions and operation (mm Hg)
Decerebration	5	105 ± 3	155 ± 8*	100 ± 7
Cerebellectomy	5	108 ± 4	152 ± 6*	158 ± 5†
Adrenonephrectomy	4	100 ± 3	152 ± 2*	149 ± 2‡

All values are means ± SE. Control values refer to prelesional blood pressure taken 30 minutes after anesthesia was discontinued. The rat was reanesthetized and bilateral lesions of the NTS were made. Anesthesia was then discontinued and blood pressure was measured 30 minutes later. The rat was once again anesthetized, the indicated operation was performed, and 30 minutes after discontinuation of anesthesia the blood pressure was again measured.

*Values differ from control values ($P < 0.001$).

†Values differ from control values ($P < 0.001$) but not from values after NTS lesions.

NTS lesions or, if performed before the NTS lesions were made, blocked the predictable development of hypertension (Table 2). This finding demonstrates that the lowering of blood pressure by decerebration in rats with NTS lesions was not the result of a nonspecific effect of cerebral injury or hemorrhage. Rather the finding indicates that the integrity of rostral brain areas was essential for the expression of the hypertension.

Effects of Lesions of the Nucleus Tractus Solitarius on Baroreceptor Reflexes.—Since lesions of the NTS in cats anesthetized with chloralose abolish the reflex hypotension and bradycardia evoked by stimulation of baroreceptors, we sought to determine if similar lesions in rats would also abolish the reflexes. The cardiovascular component of the baroreceptor reflexes, evoked by infusion of either norepinephrine (0.05–0.2 μ g in 0.2 ml) or angiotensin II (0.05–0.2 μ g in 0.2 ml), was examined in five rats lightly anesthetized with alpha-chloralose (30 mg/kg, iv). Injection of 0.05–0.2 μ g of either drug elicited reflex bradycardia associated with hypertension (Fig. 5). Before NTS lesions, all rats showed a typical graded bradycardia. After the lesions, the reflex bradycardia was completely abolished in all rats, and sometimes the heart rate response was reversed to tachycardia. These findings indicate that the NTS lesions effective in producing hypertension abolished baroreceptor reflexes.

Natural History of Hypertension.—All rats with adequate bilateral lesions of the NTS died within 8 hours. Before death the rats had labored breathing, audible gurgling rales, wheezes, and occasionally pink frothy fluid in their nostrils. At postmortem examination the rats had boggy lungs with frothy fluid often filling the trachea and bronchi. In most

rats the lungs were characterized by moderate to intense interstitial edema, especially perivascular edema, congestion, and partial atelectasis. Intra-alveolar edema was often seen.

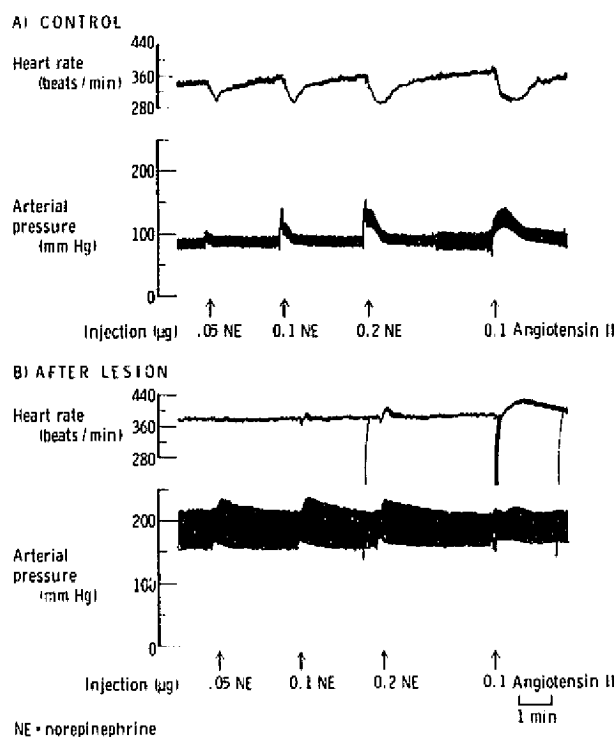


FIGURE 5

Reflex bradycardia and systemic hypertension evoked by intravenous injection of different doses of norepinephrine (NE) and angiotensin II before (A) and after (B) production of bilateral lesions of the NTS in the anesthetized rat (alpha-chloralose 30 mg/kg, iv). Before lesions, both agents produced a graded bradycardia associated with hypertension. After lesions, the reflex bradycardia was no longer elicited, and tachycardia and arrhythmias were observed with injections of norepinephrine and angiotensin II.

To determine the nature of the cardiovascular events leading up to the development of the pulmonary edema, we followed the change in blood pressure and heart rate in 11 rats from the time of lesioning until death. All had indwelling catheters in their ventral tail arteries. A representative example of this experiment is shown in Figure 6. After reaching a hypertensive plateau the systolic blood pressure gradually declined in association with a smaller drift in the diastolic pressure and a narrowing of the pulse pressure. During this period there was a small reduction in heart rate. Blood pressure fell precipitously 3–4 hours later, the clinical signs of pulmonary edema suddenly appeared and death ensued. All 11 rats died spontaneously within 5 hours. Ten rats had clinical evidence of pulmonary edema shown by their increased lung weight–body weight ratio (1.17 ± 0.11) which was significantly different from that of 12 normal control rats (0.77 ± 0.12 , $P < 0.01$) killed by an overdose of sodium pentobarbital (80–100 mg, ip).

Discussion

PERIPHERAL MECHANISMS

This study demonstrated that bilateral lesions of the NTS at the level of the obex in rats invariably resulted in the appearance of a syndrome of acute arterial hypertension. The elevation of blood pressure began almost immediately after placement of the lesions and discontinuation of anesthesia. The hypertension was neurogenic and resulted from an

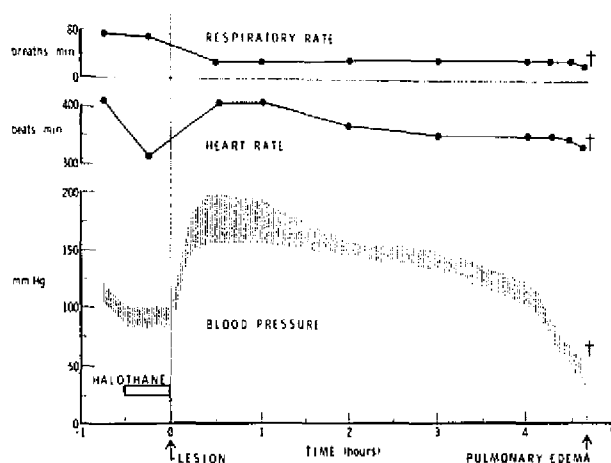


FIGURE 6

Time course of changes in systemic arterial blood pressure, heart rate, and respiratory rate in a representative unanesthetized rat after production of bilateral lesions in the NTS. Just prior to death the rat developed pulmonary edema. See text for details.

increased peripheral vascular resistance, which was due to intensive vasoconstriction secondary to augmented discharge of sympathetic preganglionic neurons. A similar mechanism also underlies the hypertension evoked by baroreceptor denervation in the rat (8). The vasoconstriction in our rats was probably mediated by alpha receptors, since the hypertension was blocked by the administration of phentolamine (30). Humoral agents released from the kidneys or the adrenal glands did not significantly contribute to the syndrome, at least not in the acute stage, since the magnitude of hypertension was unaltered by the removal of these organs.

The discharge of sympathetic preganglionic fibers was differentiated, not generalized, since there was no evidence of any associated mydriasis, proptosis, or hyperhidrosis at the time of maximal sympathetic engagement of the circulation. In addition to constriction of the resistance vessels, the capacitance vessels were probably engaged, since there was increased central venous pressure. The elevation of venous pressure, however, might be secondary to the reduced cardiac output and the congestive heart failure.

The absence of tachycardia could mean that the cardiac chronotropic sympathetic fibers were not activated by the lesion, implying a further dissociation of sympathetic nervous activity to blood vessels and heart. However, the absence of tachycardia could be due to other mechanisms including subsensitivity of the cardiac pacemaker resulting from heart failure or destruction of brainstem nuclei adjacent to the NTS in the parhypoglossal area, which might be necessary for the expression of changes in heart rate (26).

Paralleling the development of arterial hypertension was an immediate and marked reduction in cardiac output. The fall in cardiac output appeared to be the result of a reduction in stroke volume in response to the increase in total peripheral resistance. The decreased cardiac output was reflected in the elevated left ventricular end-diastolic pressure. The blockade of vasoconstriction with phentolamine reversed the left ventricular end-diastolic pressure to normal. The fall in cardiac output resulting from the increased afterload was further aggravated by the absence of compensatory tachycardia. Over a period of hours, cardiac output appeared to drop further as suggested by the disproportionate rate of decrease in systolic pressure and the gradual reduction of heart rate. Preterminally, the blood pressure dropped to shock

levels without tachycardia. Whether the rapid fall in blood pressure reflected forward failure due to a sudden dilatation of peripheral vessels, possibly secondary to local acidosis, or was the result of myocardial failure remains to be determined. The rapid decline in blood pressure and presumably cardiac output preceded the terminal pulmonary edema, suggesting that the pulmonary edema was the result of left ventricular failure. An increase in circulatory volume as a consequence of vasoconstriction of the capacitance vessels or neurogenically mediated changes in the pulmonary circulation conceivably could also contribute to the pulmonary edema (31).

CENTRAL MECHANISM

The syndrome of acute neurogenic hypertension probably resulted from the release of preganglionic sympathetic neurons from the inhibition by arterial and intracardiac stretch receptors (32) and was not due to any irritative (i.e., stimulatory) effect of the lesion. The arguments in support of this conclusion are several. First, the critical site in the brainstem which had to be damaged bilaterally to produce this syndrome was the middle third of the NTS located at the obex, the so-called intermediate zone of the nucleus (11). Through this region afferent fibers from baroreceptors are funneled, and many terminate here (14). Lesions in this area abolish the reflex hypotension and bradycardia evoked by stimulation of baroreceptors or intracardiac receptors (17). Hence damage to this area of the NTS is adequate to interrupt baroreceptor input to the brain.

Second, the reflex blood pressure response to stimulation of almost all vascular stretch receptors is a fall in systemic blood pressure due to the inhibition of sympathetic outflow (33). Withdrawal of such afferent inputs by transecting the carotid sinus and the aortic depressor nerves results in a rise in blood pressure due to an increase in sympathetic activity (10). The effects of bilateral lesions of the NTS on blood pressure are thus those that would be predicted by the hypothesis that neurogenic hypertension in the rat is due to functional deafferentation of the input of vascular stretch receptors to the brain.

Third, if the hypertension were due to irritation of the NTS, then electrical stimulation of the area would also produce hypertension. Electrical stimulation of the NTS, however, produces a fall, not a rise, in blood pressure (34).

Finally, the syndrome of hypertension is qualitatively similar in most respects to that produced by sinoaortic denervation in the rat (8, 9, 35) and other species. It is neurogenic, results in increased peripheral vascular resistance, requires bilateral lesions, and is not associated with widespread activation of sympathetic fibers. Also, it is sensitive to anesthetics and decerebration. It differs from hypertension produced by sinoaortic lesions principally in its intensity. The greater magnitude of the hypertension elicited by lesions of the NTS probably relates to the fact that the central lesions interrupt depressor reflexes mediated by the vagus and by the sinoaortic nerves.

One of the most characteristic features of the hypertension evoked by NTS lesions was its dependence on the integrity of structures lying above the midbrain. Midcollicular decerebration aborted the development of hypertension before NTS lesions or abolished hypertension once NTS lesions had been established. The importance of the rostral regions of the brain in mediating the hypertension parallels the observation of Reis and Cuenod (36) and of Manning (37) that the reflex hypertension in cats elicited by sinoaortic denervation or carotid occlusion is abolished by decerebration. Our findings suggest that baroreceptors, after terminating in the medulla (11-17) engage in long-loop cardiovascular reflexes with higher brain areas. Also, they support the view that the pressor response to restoration of baroreceptor activity is subserved by neurons different from those mediating the responses to baroreceptor excitation (36). The precise localization of the rostrally situated regions necessary for the hypertension remains to be established: conceivably it lies within the hypothalamus. Baroreceptor activity projects to the hypothalamus along polysynaptic pathways (38-40), and electrical stimulation of the hypothalamus modifies baroreceptor reflexes (41, 42). Recently, Thomas and Calaresu (40) have described a restricted zone of the posterior medial hypothalamus in the cat where electrical stimulation produces hypertension and tachycardia and where unit activity can be modified by electrical stimulation of systemic baroreceptors. This critical rostral region conceivably could be a site of interaction between behaviorally determined cardiovascular events and baroreceptor reflexes.

To our knowledge the syndrome of acute fulminating neurogenic hypertension elicited by bilateral lesions of the NTS has never been described previously. Indeed, acute fulminating

hypertension as a consequence of focal brain lesions is extremely uncommon in experimental neurology. Most reports relate to clinical cases arising from brainstem encephalitis (43-46), a relatively widespread process. However, the exquisite sensitivity of this form of hypertension to anesthesia and its dependence on the integrity of areas above the midbrain probably explains why it has not been seen before in animal studies in which the NTS has been lesioned bilaterally. However, the pulmonary edema in the unanesthetized guinea pig produced by bilateral lesions of the vagal nucleus reported by Borison and Kovacs (24), who did not measure blood pressure, possibly is similar to the syndrome we have described in this paper. The bilateral lesions of the vagal nucleus which they published also revealed damage to the NTS thereby raising the question of whether other studies in which pulmonary edema was produced by manipulation of the floor of the fourth ventricle might have also resulted from bilateral damage to the NTS (47).

Acknowledgment

Jana Iglich and Nancy Shieh provided excellent technical help.

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Reprinted from
23 November 1973, Volume 182, pp. 845-847

SCIENCE

Predatory Attack, Grooming, and Consummatory Behaviors Evoked by Electrical Stimulation of Cat Cerebellar Nuclei

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Predatory Attack, Grooming, and Consummatory Behaviors Evoked by Electrical Stimulation of Cat Cerebellar Nuclei

Abstract. *Electrical stimulation at single sites in the rostral fastigial nucleus elicits hypertension, grooming, feeding, and attack behaviors in the cat. The stimulus intensity and availability of suitable goal objects determines the behavior. Bilateral lesions of the area fail to produce motor deficits. The rostral fastigial nucleus may be a cerebellar area for behavioral and autonomic regulation.*

Traditionally, the fastigial nucleus has been viewed as sharing in the general function of the whole cerebellum in the regulation of movement and posture (1). However, there have been reports that electrical stimulation in or near the fastigial nuclei may produce cardiovascular (2) and even behavioral (3) responses. Several questions have been raised by these observations. Does the fastigial nucleus participate in the regulation of visceral and behavioral activities? If so, are such activities independent of the motor function of the nucleus? And, finally, are there anatomically distinct areas of the nucleus that mediate these activities?

Electrical stimulation of the ventromedial portion of the rostral pole of the fastigial nucleus in several species elicits a highly reproducible, stereotyped, and differentiated activation of the sympathetic nervous system (4-6). The response, termed the fastigial pressor response (5), is characterized by a marked elevation of blood pressure, heart rate acceleration, and vasoconstriction, an autonomic pattern simulating the reflex cardiovascular responses to assumption of an upright posture (6). The fact that such stimulation fails to produce evident changes in motor activity (4, 5) raises the possi-

bility that this region of the fastigial nucleus may influence visceral activity apart from its participation in somatomotor regulation.

To further evaluate the function of this area of the fastigial nucleus, we examined the effect of electrical stimulation and small lesions in unanesthetized cats on behavioral and motor performance and the relation of these responses to evoked activity. Our results suggest that a restricted portion of the fastigial nucleus may be primarily involved in visceral and behavioral control.

Nine adult mongrel cats of both sexes were studied; it had been shown before surgery that these animals did not spontaneously kill rats. The animals were anesthetized with α -chloralose (50 to 60 mg per kilogram of body weight, intravenously). Under sterile conditions, a Silastic rubber cannula was inserted into the common carotid artery, brought out through a stab wound in the back of the neck, and connected to a strain gauge transducer for blood pressure and heart rate recording by standard methods. Stainless steel electrodes, insulated to within 0.5 mm of the tip, were then inserted stereotaxically into regions of the fastigial nucleus from which blood pres-

sure responses could be elicited (5, 6). After the electrodes were implanted, the incision was closed and the cannula was plugged.

Several days later, when fully recovered from anesthesia and surgery, the animals were placed in an observation cage. The arterial cannula and wires from the stimulation electrodes were attached to one end of a swivel device located on top of the cage. The strain gauge transducer and wires from a constant current stimulator were connected to the other end of the swivel. In each session, we examined the effects of graded electrical stimuli on behavioral and cardiovascular responses. Various combinations of animal chow, water, and live or dead rats were used for goal objects in the behavioral tests. Animals were observed for several weeks. After a suitable number of testing sessions, a lesion was made at each electrode site by passage of an anodal constant current (150 μ a for 40 seconds). Over the ensuing days, the animals were tested for changes in the evoked autonomic and behavioral responses. In addition, each animal was carefully examined for abnormalities of posture and gait, disturbances of visual and tactile placing, hopping and deep tendon reflexes, and changes in the defensive responses to tail pinch or attack by another cat. After 1 to 2 weeks of testing, the animals were anesthetized with pentobarbital (60 mg/kg, intravenously) and perfused through the aorta with 10 percent formalin for subsequent histological identification of lesion sites.

Electrical stimulation of the rostral fastigial nucleus in these unanesthetized cats produced a prompt elevation of the systolic and diastolic arterial blood

pressure and an elevation of the heart rate (Fig. 1). Electrical stimulation (7), at the threshold for cardiovascular responses did not produce any overt changes in behavior (Fig. 1A). Stimuli strengths of 1.2 to 1.5 times the threshold for the cardiovascular responses elicited marked behavioral changes

(Figs. 1 and 2). At the threshold for behavioral activation, the animal would alert and, if reclining, sometimes rise to a sitting or standing position (Fig. 2A). Slightly higher stimulus intensities produced increased cardiovascular responses (Fig. 1B) and stimulus-bound grooming in all animals which was in-

distinguishable from the spontaneous behavior.

At still higher stimulus intensities, feeding and even larger cardiovascular responses were evoked in five of the nine cats (Figs. 1C and 2B). The animal approached, energetically chewed, and swallowed food placed nearby, and occasionally lapped water.

The behavior with the highest threshold was predatory attack and was elicited in seven of nine animals (Figs. 1D and 2D). Attack on a live or dead rat had a latency of several seconds. The animal would visually fix on the prey, growl or intensely meow, crouch (Fig. 2C), and suddenly bite the head and neck of the rat (Fig. 2D). The cat did not assume any of the postures or autonomic stigmata (such as pupillary dilatation or piloerection) characteristic of the defense reaction (8). The biting attack was savage and would persist during stimulation. If stimulation was repeated, the animal would, after killing the prey, devour its head, tail, and paws. In five of the nine animals, all three of these behavioral patterns (grooming, feeding, and attack) were evoked from a single electrode site.

The determinants of which behavior was elicited were related to stimulus intensity and, in part, to the availability of the goal object. Thus, if a rat and food were both available, stimulation at intensities eliciting killing would produce only that behavior. The animal would eat after removal of the rat and would groom after removal of food. In two cats, however, the thresholds for attack and eating overlapped. The animals would preferentially attack if both food and prey were available. In the absence of prey, the animals would eat. All cats showed an increased propensity for biting or chewing any object placed near or touching the mouth.

Stimulation at sites from which behavior was evoked always elicited cardiovascular changes. Conversely, stimulation at any site from which a cardiovascular effect was elicited always evoked a behavior. However, cardiovascular changes were not essential for the appearance of evoked behaviors. Blockade of the pressor response by phentolamine (1 mg/kg, intravenously) did not affect the threshold or form of the behavioral responses.

Stimulation of the ventromedial pole of the fastigial nucleus evoked changes in posture or movement that were al-

Fig. 1. Relation between stimulus intensity, cardiovascular response, and behavior elicited by electrical stimulation at the same site in the rostral fastigial nucleus of an individual cat. In each frame the upper trace is blood pressure recorded from the carotid artery, the middle tracing is mean blood pressure, and the lower tracing is instantaneous heart rate. The black bar represents the stimulus train (50 hertz for 40 seconds). The arrow represents onset of designated behavior. Increasing the stimulus intensity elicits different behaviors and augments the cardiovascular responses; bpm, beats per minute.

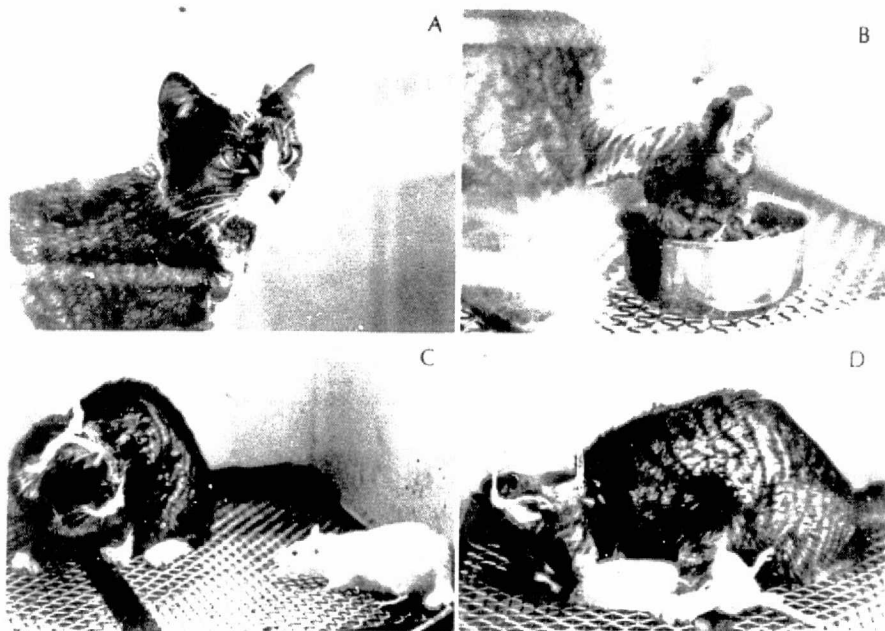
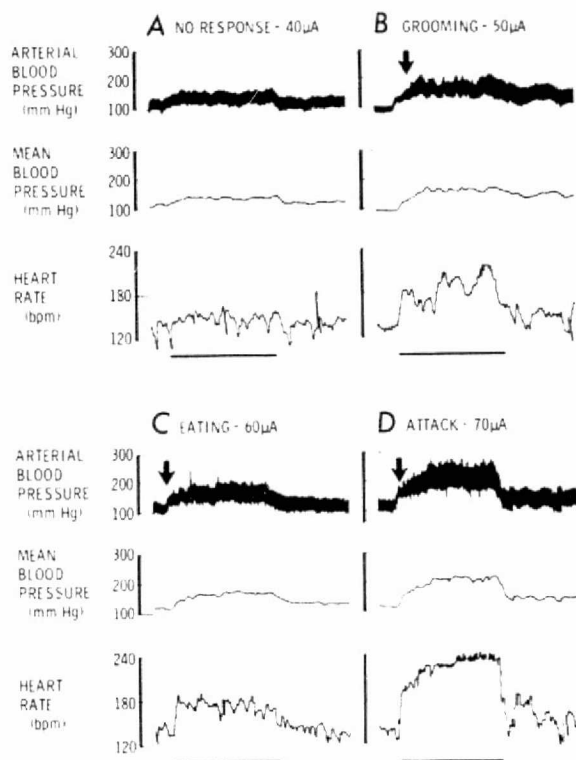


Fig. 2. Alerting (A), feeding (B), predatory (biting) attack (C), and consumption of prey after attack (D) evoked by electrical stimulation of the rostral fastigial nucleus in an individual cat. This behavioral sequence was produced by increasing stimulus intensity at a single electrode site.

ways associated with behavioral and cardiovascular responses. Electrodes placed at adjacent sites in the dentate nuclei elicited motor, but not behavioral or visceral changes.

Bilateral lesions at the effective sites in the fastigial nucleus abolished the behavioral and cardiovascular responses to stimulation. Thus, the evoked responses were not the result of current spread. A mild truncal tremor and hindlimb ataxia that disappeared 2 days after placement of the lesions was seen in only two cats. The defense response of all animals elicited by tail pinch or threat by another cat were unaffected by the lesions.

The present study demonstrates that electrical stimulation restricted to the rostral and ventromedial pole of the fastigial nucleus can elicit grooming, feeding, and predatory attack in cats, all behaviors heretofore considered to be primarily represented in the hypothalamus and limbic systems. The evoked behaviors are integrated, appropriately goal-directed, and not associated with alterations in movement or posture inappropriate for the behavior. The behaviors are specifically related to stimulation of a restricted area in the fastigial nucleus, since they cannot be elicited after coagulation around the electrode tip or by stimulation of adjacent cerebellar nuclei. While others have noted that electrical stimulation of deep cerebellar nuclei may produce emotional "behaviors" (3), the earlier studies failed to show whether such behaviors were differentiated, were independent of motor activation, or had a discrete localization. This study would, therefore, appear to be the first in which clearly defined behaviors could be evoked independently of any motor activity from a highly localized site within the cerebellum.

The range of behaviors evoked from a restricted locus in the fastigial nucleus is notable. While it is not possible by the use of fixed electrodes to ascertain whether there is anatomically discrete representation of grooming, feeding, or attack within this limited zone of the fastigial nucleus, there are several reasons to conclude that the behavior is not organized topographically. First, the fact that all three behaviors were elicited in five animals and two were elicited in seven animals with an identical gradation of stimulus thresholds suggests a considerable overlap for the representation of each behavior at a single fastigial site. Thus, it is the in-

tensity of the stimulus and not the location of the electrode which is one of the determinants of the identity of the behavior. Second, the observation that the nature of the behavior evoked from a single electrode at a fixed stimulus intensity could be changed by altering the availability of goal objects (such as food or prey) is another demonstration that the locus of the electrode is not critical. Thus, our findings suggest that the behavioral responses from fastigial stimulation are probably not due to excitation of discretely organized neural pathways. Rather, they appear to result from a general activation of neurons subserving discrete fixed-action patterns, the resultant behavior being determined, in part, by the intensity of the activation and the presence of suitable goal objects.

This interpretation of the organization of behavior within the rostral fastigial nucleus parallels and supports the view of Valenstein regarding the organization of behavior within the hypothalamus of the rat (9). It would strongly support his view that the central neural organization of some behaviors is not fixed, but is plastic and subject to environmental control.

This study has demonstrated an inextricable relation between behavioral and cardiovascular responses from electrical stimulation of the rostral fastigial nucleus. The various evoked behaviors were all associated with increased blood pressure and heart rate. The pattern of the autonomic responses elicited from this site in the rostral fastigial nucleus is stereotyped (6). Only the magnitude of the response varies as a function of stimulus strength. However, the nature of the behavioral responses depends on stimulus strength, and frequently all of the behaviors are elicited from the same stimulation site. These findings contrast with the relation between cardiovascular responses and behavior evoked from the hypothalamus in the cat, for which different evoked behaviors (such as feeding or defense) are associated with particular cardiovascular responses characteristic of the behavior (10) and depend upon the stimulation site. The stereotyped set of autonomic adjustments common to each of the different evoked behaviors from the fastigial nucleus might represent activation of the cardiovascular responses associated with assumption of an upright posture (6) possibly in anticipation of movement, a common factor

shared by all of the behaviors reported here.

It is not possible to define the pathways that mediate the behavioral responses to stimulation of the rostral fastigial nucleus. We have shown that the cardiovascular responses are relayed via the fastigiobulbar tract (5) to the paramedian reticular nucleus in the medulla and thence through as yet undefined pathways to the spinal cord. It is possible, however, that the behavioral components may be due to orthodromic excitation of other pathways projecting centrifugally from the fastigial nucleus to other brainstem nuclei or to antidromic excitation of axon collaterals projecting onto the fastigial nucleus. However, the fact that behavior and cardiovascular activity can be elicited from electrical stimulation that does not evoke any alteration in motor activity and that lesions of this restricted site of the fastigial nucleus fail to result in a motor deficit suggests that this restricted region of the cerebellum may primarily function as a modulator of emotional and visceral behaviors.

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7. The electrical stimulus was a 40-second train of square-wave pulses of 0.5-msec duration generated by a constant current stimulator at 50 hertz. The stimulus current was also monitored by an oscilloscope by simultaneously measuring, after suitable amplification, the voltage drop across a resistor attached in series between the output of the stimulator and the electrode. The usual threshold current for blood pressure responses ranged from 0.010 to 0.050 μ A.
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11. Supported by grants from NIH (NS 04876), NASA (NGR 33-010-179), and the Harris Foundation.
12. July 1973



Role of the Cerebellum and the Vestibular Apparatus in Regulation of Orthostatic Reflexes in the Cat

By Nobutaka Doba and Donald J. Reis



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Role of the Cerebellum and the Vestibular Apparatus in Regulation of Orthostatic Reflexes in the Cat

By Nobutaka Doba and Donald J. Reis

ABSTRACT

The contribution of the fastigial nucleus and the vestibular nerves (eighth cranial nerves) to the orthostatic reflexes in anesthetized, paralyzed cats was studied. Bilateral lesions of the rostral fastigial nucleus resulted in impairment of the reflex changes in blood pressure, femoral arterial flow, and resistance evoked by head-up tilting to 30° or 60°. The deficit consisted of an increase in the magnitude of the initial fall in blood pressure during tilting. The effects on blood pressure were paralleled by decreased vasoconstriction in the femoral artery. Extracranial lesions of the vestibular nerves produced comparable deficits which were not enhanced by subsequent lesions of the fastigial nucleus. Denervation of the baroreceptors impaired the reflexes, and subsequent lesion of the fastigial nucleus increased this deficit. The pressor response evoked by electrical stimulation of the rostral fastigial nucleus also reversed the deficit in orthostasis produced by hemorrhage. Small doses of sodium pentobarbital which did not alter the resting blood pressure or the pressor response to carotid occlusion impaired the responses to stimulation of the fastigial nucleus and tilting. Therefore, the rostral fastigial nucleus, which might be triggered by the vestibular apparatus, appears to participate in concert with the baroreceptors in the initiation and possibly the maintenance of the orthostatic reflexes.

KEY WORDS sympathetic nervous system blood pressure posture
cerebellar nuclei blood flow brain stimulation baroreceptor reflexes

■ The orthostatic reflexes consist of a patterned activation of sympathetic neurons in response to the assumption of an upright posture (1). The preponderant effect is a widespread vasoconstriction of resistance and capacitance vessels and tachycardia (1, 2). The vasoconstriction tends to resist the hydrodynamic action of gravity which forces the blood into the dependent extremities and viscera; the response thereby maintains blood pressure and protects circulation to the brain.

It is widely assumed that the orthostatic responses are triggered by baroreceptors, particularly those in the carotid sinus (1, 3) and possibly those in the low-pressure circulation (4-6). According to this view, the reduction in stretch of the sinus during standing results in a decrease in discharge from the baroreceptors, a release of pre-ganglionic sympathetic neurons from inhibition, and thus an increase in sympathetic nerve activation and vasoconstriction. There are, however, several studies (7, 8) in which deafferentation of

baroreceptors failed to abolish the orthostatic responses. Such findings suggest that receptors other than the baroreceptors may participate in initiating these postural reflexes.

It has recently been discovered (9-11) that electrical stimulation of a highly restricted region of one of the deep nuclei of the cerebellum, the fastigial nucleus, can elicit a powerful activation of the cardiovascular system. The cardiovascular events associated with the fastigial pressor response consist of an elevation in systemic arterial blood pressures, tachycardia, and vasoconstriction of arteries in limbs, kidney, and abdominal viscera (11-14); this cardiodynamic pattern simulates the orthostatic reflex. On this basis we have suggested (14) that the cerebellum may participate in the regulation of orthostatic mechanisms. Moreover, since the cerebellum receives neural input from the vestibular apparatus (15-17), the vestibular apparatus may also participate in the cardiovascular responses to upright posture.

In the present study we attempted to determine whether the cerebellum and the vestibular apparatus participate in initiating orthostatic reflexes and whether they interact with baroreceptors in performing this function by studying the effects of selected lesions or of electrical stimulation of the fastigial nuclei, the vestibular nerves, and the

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This work was supported by NIH Grant NS 04876 and Grant NGR 33-010-179 from the National Aeronautical and Space Administration.

Received July 13, 1973. Accepted for publication November 2, 1973.

baroreceptors on the reflex cardiovascular responses to tilting in the cat. Our findings supported the view that cerebellar and vestibular mechanisms are involved in initiating and sustaining orthostatic reflexes. Preliminary reports of this study have been published (12, 18).

Methods

Forty-two adult cats were anesthetized with alpha-chloralose (40–50 mg/kg, iv). All cats were paralyzed with gallamine triethiodide (5 mg/kg, iv) and artificially ventilated through a tracheal cannula. End-tidal CO_2 recorded by an infrared gas analyzer (Beckman L1), was maintained at 2–3%. Rectal temperature was maintained at 37°C by a thermostatically regulated infrared lamp.

A polyethylene catheter was placed in the aorta via the femoral artery, and the tip was positioned at the approximate point where the diaphragm is attached to the posterior body wall. This level corresponds to the hydrostatic indifferent point (1). The location of the catheter tip was confirmed at the end of each experiment. The systemic blood pressure was recorded from the aortic catheter by a pressure transducer (Statham P23Db), and the heart rate was computed from the blood pressure pulse by a cardi tachometer (Beckman type 9857). These records were simultaneously displayed on channels of a polygraph (Beckman Dynograph type 504A). Blood flow was recorded from the femoral artery by an electromagnetic flowmeter as previously described (14), and the vascular resistance was computed by dividing the mean arterial blood pressure by the mean arterial flow. The mean arterial blood pressure was derived from the formula $(P_s + 2P_d)/3$, where P_s is systolic pressure and P_d is diastolic pressure. After attaching flow probes to the appropriate arteries, the cat was placed in a stereotaxic frame with the head flexed to 45°.

Two electrodes consisting of Teflon-coated steel wires (diameter 0.006 inches) bared at the tip for 1 mm and carried in no. 28 stainless-steel hypodermic tubing (14) were placed separately into each fastigial nucleus through a small burr hole in the calvarium just above the nuchal ridge. To identify the optimal placement in the fastigial nucleus, the electrodes were lowered to be the cerebellum initially in 0.5-mm steps, and monopolar stimulation was applied; the indifferent electrode was a copper clip placed on the temporalis muscle. The brain was stimulated with square-wave pulses (0.1–0.2 ma, 50 Hz, 0.1-msec pulse width) generated by a constant-current stimulator until a site was reached from which an optimal pressure response was evoked (9). The electrodes were then firmly secured to the skull with dental cement. Later in the experiment, electrolytic lesions were made through these electrodes.

After implantation of the electrodes, the cat was placed on a tilt table with a suitable saddle and secured by ligatures tied to the forelimbs. The head and neck were firmly fixed to the table thereby preventing their

movement during tilting. The axis of tilt of the table passed through the approximate point of the tip of the catheter in the aorta. The zero reference line of a pressure transducer was placed at the level of the axis of tilt. Thus, the zero level was constant at all times during tilting. Pressure recorded in this way represents central perfusion pressure (1). The cat was then tilted to 30° and 60° in a head-up position off the horizontal over 2–3 seconds. Within this time there was no appreciable alteration in systemic blood pressure (7). The upright posture was maintained for exactly 1 minute, and then the cat was returned to the horizontal position over 2–3 seconds. After obtaining base-line observations on the cardiovascular responses to tilting, the cat was subjected to various experimental procedures including placement of electrolytic lesions in the fastigial or other deep cerebellar nuclei, electrical stimulation of the fastigial nuclei, denervation of the vestibular nerves or peripheral baroreceptors, and intravenous administration of drugs. A minimal interval of 30 minutes was allowed following placement of lesions and retesting of the cardiovascular responses.

Electrolytic lesions of the cerebellar nuclei were made by passing a d-c anodal current (5 ma) for 30–45 seconds from a constant-current d-c source through the implanted electrodes. In 11 cats, lesions were placed bilaterally in the fastigial nuclei at the pressor sites. In 8 cats, extrafastigial cerebellar lesions were produced in or near the adjacent interpositus nuclei. In 8 cats, the vestibular nerve was denervated extracranially. The tympanic bullae were exposed ventrally through the neck; the mastoid processes were exposed by midline cervical incision and opened with an electric drill. With careful dissection, the peripheral branches of the vestibular nerve innervating the semicircular canals and the utricle would be visualized along with part of the basal portion of the cochlea. These branches were transected with small iris scissors. In 6 cats, the peripheral baroreceptors were denervated by transecting both the carotid sinus nerves and the vagi at the midcervical level. In some experiments, the response to carotid occlusion was determined by occluding the common carotid arteries bilaterally with a small arterial clamp for 12 seconds. When electrical stimulation was combined with the tilt procedures, the fastigial nucleus was stimulated with a 1–2-minute train of 0.1-msec square-wave pulses at a stimulus frequency of 10 Hz.

At the end of each experiment, the cat was killed by perfusing the heart with 10% formalin (w/v). The brain was removed and fixed, and frozen sections were stained for cells by Nissl's method to identify the lesion sites.

The significance of changes in cardiovascular parameters was estimated by a paired *t*-test; $P < 0.05$ was significant.

Results

CARDIOVASCULAR RESPONSES TO HEAD-UP TILTING IN THE ANESTHETIZED, PARALYZED CAT

The reflex cardiovascular responses to head-up

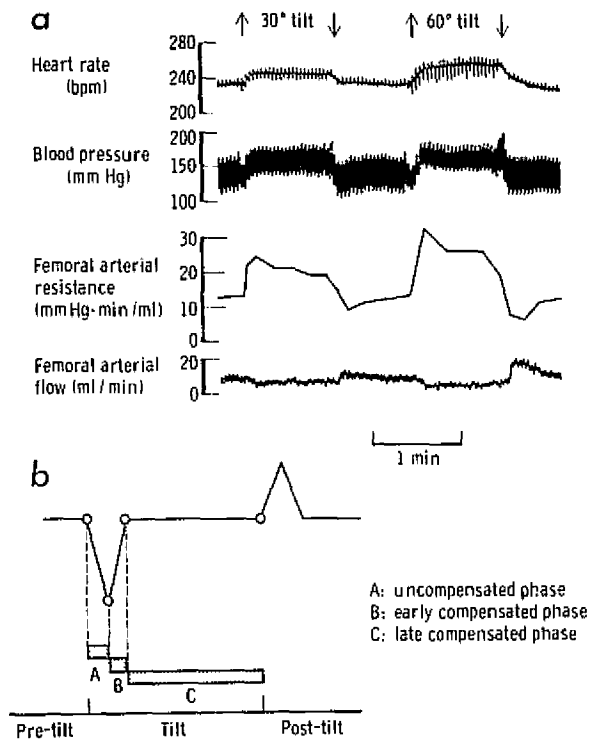


FIGURE 1

Cardiovascular responses to a head-up tilt in the anesthetized, paralyzed cat. **a**: Typical tracing showing changes in heart rate, systemic arterial blood pressure, femoral arterial resistance, and femoral arterial flow during 1 minute of head-up tilting to 30° or 60°. The onset of tilting is signified by the upward arrow, the termination is signified by the downward arrow. Note that the vascular responses are graded. **b**: Schematic representation of the phases of the blood pressure response to tilting. See text for details.

tilting are well preserved in a cat anesthetized with chloralose (7). The most common blood pressure response to a 1-minute tilt consisted of a brief fall followed by a rapid recovery (Fig. 1a). Blood pressure was then sustained at control or slightly elevated levels during the remainder of the tilt. At the cessation of the tilt, there was a characteristic brief overshoot of the blood pressure. The reflex blood pressure response can be schematically depicted in three phases (Fig. 1b): (1) an early, uncompensated phase corresponding to the initial fall in pressure, (2) an early, compensated phase when blood pressure is recovering, and (3) a late, compensated phase when blood pressure is sustained at or very near control blood pressure levels.

The maintenance of blood pressure was associ-

ated with tachycardia and the prompt onset of vasoconstriction in the femoral arterial bed resulting in a decrease in blood flow and an increase in vascular resistance (Fig. 1a) in that artery. The rate of rise of vascular resistance was rapid.

The reflex blood pressure and the vascular responses to tilting were graded (Fig. 1a). With a tilt of 60°, the magnitude of the early fall in blood pressure was increased and the latency to the early onset of compensation was delayed in comparison with a tilt of 30°. The calculated increase in resistance in the femoral artery was also graded (Fig. 1a). Although the tachycardia associated with head-up tilting was slightly graded in individual cases, on the average it was not graded.

The typical cardiovascular responses to tilting were seen in 42 of 67 cats. In the remaining 25 cats, the blood pressure did not return to control levels during the 1-minute tilt or when tilting was repeated. These cats were not included in this study. In most of the cats for which the data were discarded, however, the diminished orthostatic response appeared to be the consequence of a reduction in blood volume, because infusion of 10–15 ml of normal saline or plasma expander (Rheomacrodex) resulted in the return of a well-developed, maintained blood pressure during head-up tilting.

EFFECTS OF PARALYZATION ON ORTHOSTATIC REFLEXES

To eliminate any effects of muscle contraction on the orthostatic reflexes, the cats were paralyzed with gallamine triethiodide (5 mg/kg, iv) and artificially ventilated. Paralysis did not produce any significant differences in the blood pressure responses to tilting to 30° but did slightly increase the magnitude of the initial fall in blood pressure during tilts of 60°. This increased fall in blood pressure could be averted by firmly binding the abdomen, indicating that it probably resulted from increased pooling of blood in the splanchnic bed as a consequence of paralysis of the abdominal muscles. These findings indicate that proprioceptors probably do not participate significantly in triggering postural cardiovascular reflexes in the anesthetized cat.

EFFECTS OF BILATERAL LESIONS OF THE FASTIGIAL NUCLEUS ON ORTHOSTATIC REFLEXES

Bilateral electrolytic lesions were focally placed within the area of the fastigial nucleus from which a pressor response was evoked by electrical stimulation (Fig. 2). Such lesions significantly impaired the cardiovascular response to tilting; a representative experiment is illustrated in Figure 3



FIGURE 2

Bilateral electrolytic lesions of the rostral and the medial portions of the fastigial nucleus in the cat. This lesion impaired the orthostatic reflex. Bar = 1 mm.

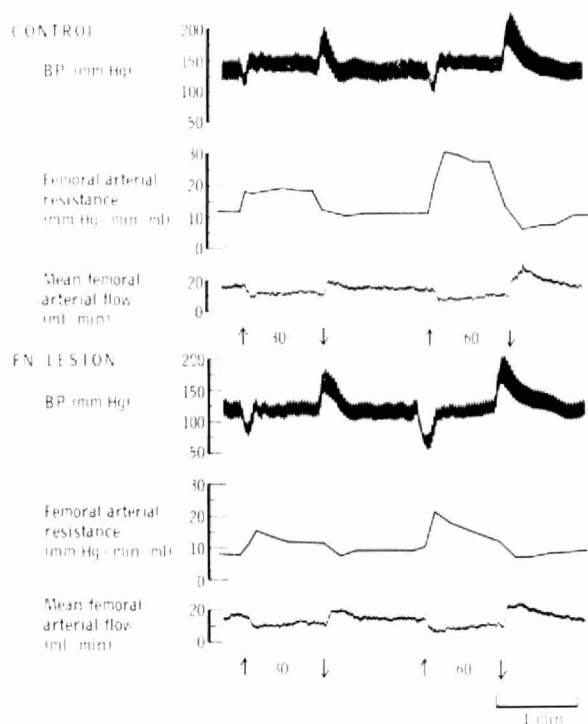


FIGURE 3

Effects of bilateral electrolytic lesions of the rostral and the medial portions of the fastigial nucleus (FN) on blood pressure (BP), femoral arterial resistance, and femoral arterial flow during tilting in the anesthetized, paralyzed cat. The cat was tilted head-up to 30° or 60° at the upward arrows and returned to the horizontal position at the downward arrows.

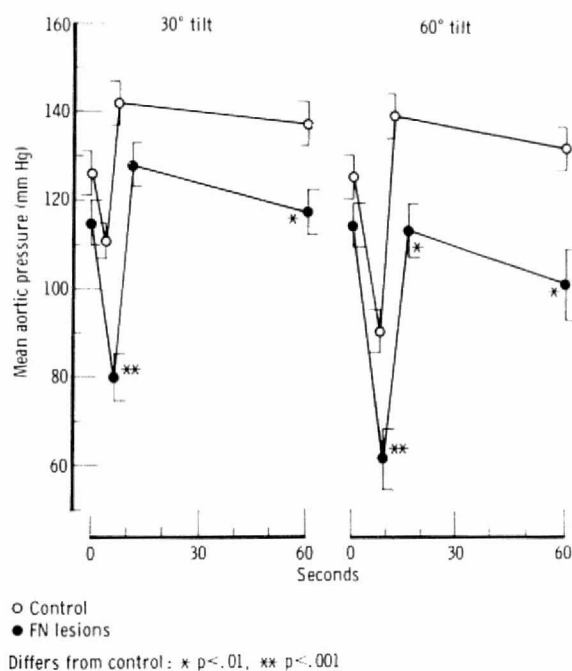
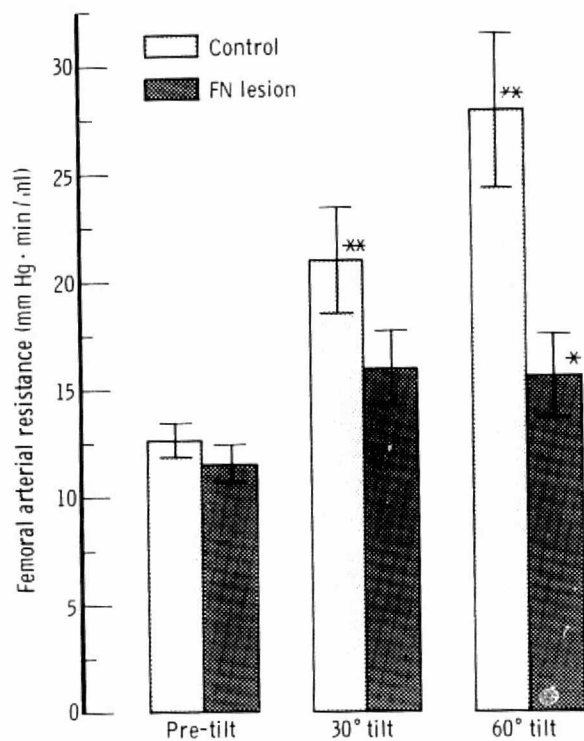


FIGURE 4

Effects of bilateral lesions of the fastigial nucleus (FN) on the mean aortic blood pressure during 1 minute of head-up tilting to 30° or 60° in 11 anesthetized, paralyzed cats. Open circles represent prelesion values and solid circles represent values after lesions had been made. Each point represents the mean \pm SE. Note the impairment in blood pressure responses produced by fastigial lesions.

and pooled data is presented in Figure 4. The deficits included (1) an augmentation of the initial fall in blood pressure (Figs. 3 and 4), (2) a delay in the onset of the early, compensated phase (Figs. 3 and 4), and (3) a failure during the late, compensated phase of blood pressure to return to control values (Fig. 4), particularly with a tilt of 60°.

The collective impairments in the blood pressure responses to tilting after fastigial lesions were probably the result of a decrease in vasoconstriction (Fig. 3) which was reflected by a delay in the onset of arterial resistance, a slower rise to peak, and a failure in the maintenance of the reflex increase in arterial resistance. The changes in vascular resistance produced by the brain lesion were consistent and surprisingly similar in magnitude from cat to cat (Fig. 5). Despite the impairment of vascular responses, the lesions did not alter the reflex tachycardia.



(n = 11)

* Differs from control $p < .01$

** Differs from pre-tilt $p < .01$

FIGURE 5

Effects of bilateral lesions of the fastigial nucleus (FN) on the changes in femoral arterial resistance elicited by head-up tilting to 30° and 60° in 11 anesthetized, paralyzed cats. The resistance was measured at the nadir of the initial hypotensive response to tilting.

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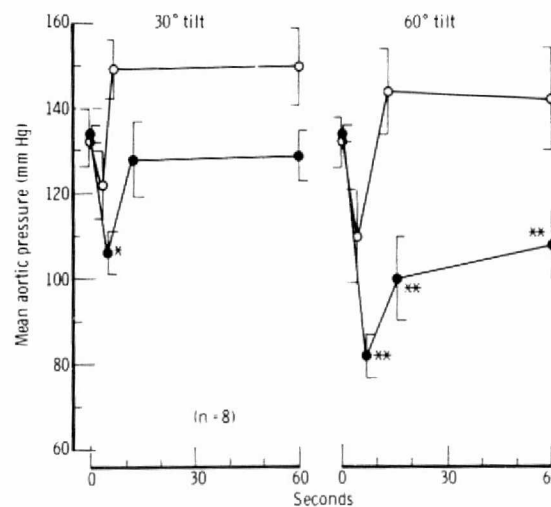
EFFECTS OF EXTRAFASTIGIAL LESIONS OF CEREBELLAR NUCLEI ON ORTHOSTATIC REFLEXES

To determine the anatomical specificity of fastigial lesions, bilateral lesions were placed in the interpositus nucleus and adjacent deep cerebellar nuclei in eight cats. Such lesions had no effect on the blood pressure response to tilting, thereby demonstrating the anatomical specificity of the rostral fastigial nucleus in the regulation of the orthostatic reflexes.

EFFECTS OF DENERVATION OF THE VESTIBULAR NERVES ON ORTHOSTATIC REFLEXES

Because the fastigial nucleus receives information from the vestibular apparatus (15-17), the fastigial mechanisms involved in orthostatic reflexes might be triggered by vestibular stimuli. To test this possibility, the effects of denervating the vestibular nerves on the blood pressure responses to tilting in eight cats were examined. As seen in Figure 6, bilateral extracranial lesions of the vestibular nerves significantly impaired the responses to tilting. The deficits were most evident during the initial phase of a 30° tilt and throughout all phases of a 60° tilt.

The impaired blood pressure responses to tilting produced by vestibular nerve lesions did not differ



○ Before denervation of VIIIth nerve

● After denervation of VIIIth nerve

Differs from control: * $p < .05$, ** $p < .01$

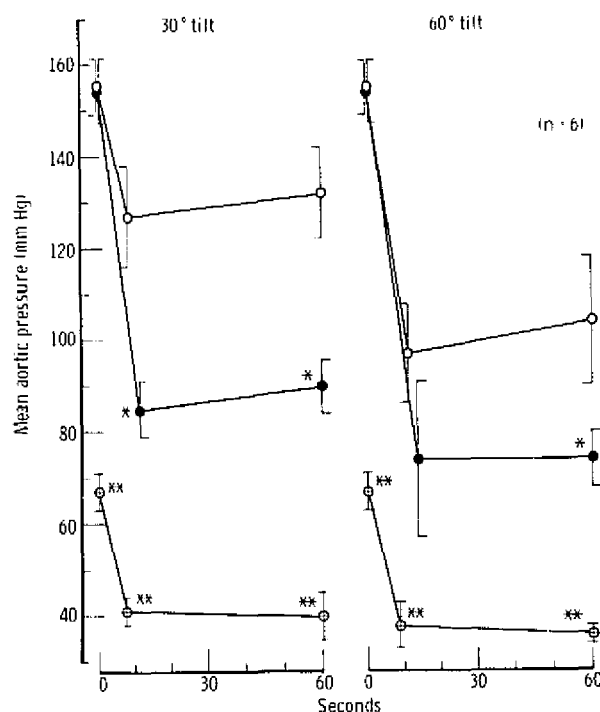
FIGURE 6

Effects of bilateral extracranial denervation of vestibular nerves (VIIIth nerve) on mean aortic blood pressure response to graded tilting in eight anesthetized, paralyzed cats. The control responses represent the responses to tilting after opening of the tympanic bullae but prior to transection of the vestibular nerves and do not differ from the responses in other unoperated controls (see Fig. 3).

from those produced by fastigial nucleus lesions alone. Moreover, in three cats, the subsequent placement of bilateral lesions in the fastigial nucleus after bilateral vestibular nerve transection did not produce any further deficit. Thus, it appears that fastigial and vestibular projections involved in the orthostatic reflexes share some common neuronal mechanism.

INTERACTION OF BARORECEPTORS WITH FASTIGIAL MECHANISMS

To examine the effects of denervation of baroreceptors on responses to tilting, either alone or in combination with lesions of the fastigial nuclei, the carotid sinus and the vagus nerves were bilaterally transected in six cats. Such denervation resulted in a sustained elevation of blood pressure (Fig. 7) as previously described (13). Head-up tilting to 30° resulted in a fall in blood pressure which failed to show any compensation over the 1-minute



- Baroreceptor denervation
- Combined baroreceptor denervation and bilateral FN lesions
- x Phentolamine, 1.0 mg/kg, iv

Differs from baroreceptor denervation: * $p < .01$, ** $p < .001$

FIGURE 7

Effects of sequentially denervating baroreceptors, lesioning the fastigial nuclei (FN), and administering phentolamine (1 mg/kg, iv) on the mean aortic blood pressure response to graded head-up tilting in six anesthetized, paralyzed cats. See text for details.

period. However, the mean blood pressure which was maintained during the tilt in baroreceptor-denervated cats was the same as that observed prior to buffer nerve transection. With a 60° tilt, the fall in blood pressure was greater, reaching levels that were slightly lower than control levels.

Bilateral fastigial nucleus lesion in cats whose buffer nerves were sectioned did not alter the elevated mean blood pressure but did cause an impairment in the compensatory response to tilting greater than that produced by a buffer nerve transection alone (Fig. 7). This finding indicates that the baroreceptor and fastigial mechanisms are additive in initiating or sustaining orthostatic reflexes, suggesting that they are subserved, at least in part, by separate neuronal mechanisms.

Even after the transection of baroreceptors and the placement of bilateral lesions in the fastigial pressor areas, blood pressure during tilting was sustained within the physiological range. The subsequent administration of phentolamine (1 mg/kg, iv), however, resulted in a prompt fall in mean blood pressure and the virtual disappearance of any compensatory responses to tilting (Fig. 7). This finding suggests that, in addition to baroreceptor and vestibular-fastigial control, mechanisms possibly of spinal origin participate in mediating the reflex blood pressure responses to tilting.

EFFECTS OF ELECTRICAL STIMULATION OF THE FASTIGIAL NUCLEUS ON THE RESPONSES TO BILATERAL CAROTID OCCLUSION

To determine the interaction between the fastigial pressor response and the orthostatic reflexes the effect of electrical stimulation of the pressor area of the fastigial nucleus on the pressor response to bilateral carotid occlusion was ex-

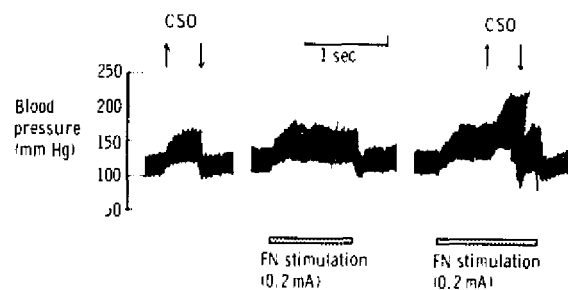


FIGURE 8

Pressor responses to carotid sinus occlusion (CSO) and electrical stimulation of the fastigial nucleus (FN) alone and in combination in the anesthetized, paralyzed cat. Bilateral common carotid arteries were briefly occluded at the upward arrows and released at the downward arrows. The fastigial nucleus was stimulated with a train of 0.1-msec square-wave pulses at a stimulus frequency of 10 Hz.

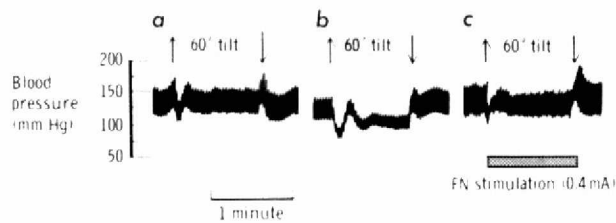


FIGURE 9

Effects of electrical stimulation of the fastigial nucleus (FN) on an impaired orthostatic reflex response produced by hypovolemia in the anesthetized, paralyzed cat. **a:** Control tilt. **b:** Tilt response 15 minutes after withdrawal of 15 ml of blood. **c:** Combined stimulation of fastigial nucleus (FN) with tilting immediately after withdrawal of 15 ml of blood. The fastigial stimulus was a train of square-wave pulses (50 Hz at 0.4 mc). Note that the impaired orthostatic response induced by hypovolemia is corrected by simultaneous stimulation of the fastigial nucleus.

aminated. The pressor responses to both carotid occlusion and stimulation of the fastigial nucleus summated (Fig. 8). This observation further indicates that the fastigial and the baroreceptor mechanisms involved in orthostatic control are, in part, distinct.

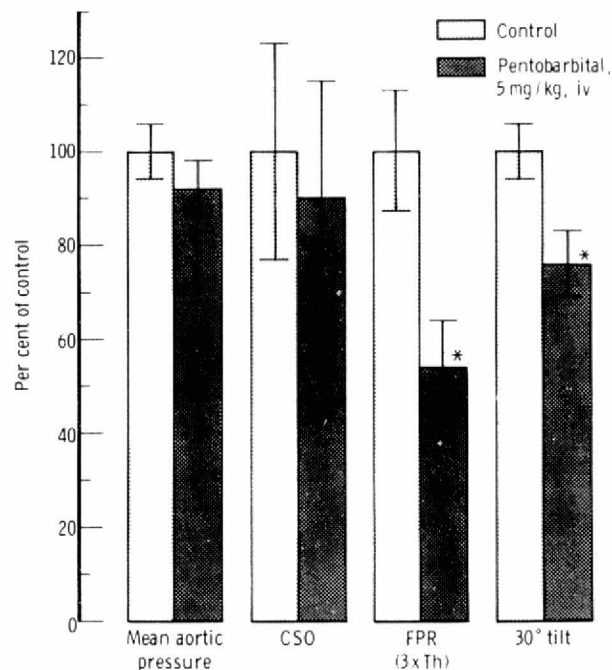
EFFECTS OF ELECTRICAL STIMULATION OF THE FASTIGIAL NUCLEUS ON DEFICIENT ORTHOSTATIC REFLEXES

The previous studies have demonstrated that lesions of the fastigial nucleus will impair the orthostatic reflexes. We also attempted to determine whether electrical stimulation of the fastigial nucleus at the pressor region could reinforce an impaired orthostatic reflex and revert it to normal. The orthostatic reflex was decompensated in four cats by acute withdrawal of 10–15 ml of blood. A typical experiment is illustrated in Figure 9. Following the withdrawal of blood, the usual orthostatic reflex response elicited by a head-up tilt of 60° (Fig. 9a) was converted to an uncompensated response (Fig. 9b). The abnormal response was entirely restored to normal (Fig. 9c) by coupling the hemorrhage with concurrent fastigial stimulation. The intensity of stimulation of the fastigial nucleus required for compensation was always above threshold for a pressor response in the supine cat. However, the observations demonstrate that fastigial excitation can serve to reinforce and compensate deficient orthostatic reflexes.

EFFECTS OF SODIUM PENTOBARBITAL ON THE BLOOD PRESSURE RESPONSES TO TILTING

Finally we attempted to determine whether the fastigial pressor mechanisms participate in the tilting responses in the otherwise intact cat by examin-

ing the effects of small doses of sodium pentobarbital (5 mg/kg, iv) on the responses to tilting, the mean aortic blood pressure, and the pressor response to withdrawal of baroreceptor input as evoked by brief bilateral occlusion of the common carotid artery. This dose of sodium pentobarbital will impair the fastigial pressor response (10) (Fig. 10). Sodium pentobarbital did not alter the mean aortic blood pressure or the magnitude of the response to bilateral carotid occlusion. However, it reduced the fastigial pressor response to 54% of the control level and also reduced the blood pressure response to tilting by (1) increasing the magnitude of the initial fall, (2) delaying the onset of compensation, and (3) failing to maintain pressure at normal levels during the tilt (Fig. 10). These findings indicate that the impairment of the



(n = 4)

* Differs from control $p < .05$

FIGURE 10

Effects of sodium pentobarbital (5mg/kg, iv) in four anesthetized, paralyzed cats on the mean aortic pressure, the maximal pressor response to bilateral carotid sinus occlusion (CSO), the maximal pressor response (FPR) elicited by electrical stimulation of the fastigial nucleus (3 times threshold, 10-second train, 50 Hz) and the mean aortic pressure during the maintained phase of head-up tilting to 30°. All values represent mean aortic pressure expressed as a percent of the control value before drug administration. Note that this dose of the barbiturate significantly reduces the magnitude of the fastigial pressor response and results in a fall in blood pressure during tilting but does not affect the resting mean aortic pressure nor the magnitude of the pressor response to carotid sinus occlusion.

tilting response parallels the reduction of the fastigial pressor response produced by a barbiturate that has no effect on the reflex pressor response to carotid sinus occlusion. We conclude, therefore, that in the intact cat the fastigial mechanisms participate in initiating and possibly sustaining the orthostatic reflexes in association with the baroreceptors.

Discussion

The present study has demonstrated that small bilateral electrolytic lesions of the rostral fastigial nucleus can impair the cardiovascular response to head-up tilting in the anesthetized, paralyzed cat (7). The deficit consists of a prolongation of the onset and a reduction in the magnitude of the reflex vasoconstriction. The disorder of vasoconstriction is reflected as a prolongation and enhancement of the transient fall in blood pressure at the moment of tilting and a failure of the blood pressure to return to control levels when the tilt is maintained.

The impairment in the orthostatic responses produced by the lesions cannot be attributed to nonspecific effects of brain damage, because comparable lesions placed in adjacent cerebellar sites fail to influence the response. It is also unlikely to be due to an irritative effect of the lesion, because stimulation at the same site facilitates the response. Therefore, impairment of the orthostatic response to tilting produced by lesions of the cerebellum is probably the result of damage to cells or fibers in the ventromedial portion of the rostral fastigial nucleus. These findings in conjunction with the observations that (1) electrical stimulation restricted to the same area of the fastigial nucleus produces a patterned cardiovascular response simulating orthostatic reflexes (14) and (2) such stimulation will reinforce and possibly correct the deficiency in orthostasis produced by hypovolemia strongly support the hypothesis that the fastigial nucleus modulates the reflex cardiovascular response to posture.

The precise mechanism by which the fastigial nucleus acts in response to tilting is uncertain. If one assumes that the nucleus is reflexly activated by tilting, the question then arises as to the source of the afferent information initiating the response. Since the cats in this study were paralyzed and their heads were not moved on the neck during tilting, it is unlikely that proprioceptive or cutaneous information was important.

One possibility examined in this study was that information rising in the vestibular apparatus may

serve as a stimulus. This contention has gained support from the observations that bilateral extracranial transection of the vestibular nerves impairs the tilting response to the same extent as do lesions of the fastigial nucleus alone. The fact that combined lesions of both the vestibular nerves and the fastigial nucleus did not produce a summated deficit indicates that they probably share a common neuronal pool. Whether this finding means that the presumed neurons in the rostral fastigial nucleus which excite sympathetic preganglionic neurons are activated by the vestibular apparatus remains to be established. There is, however, evidence that information arising in the vestibular organs is conveyed into the fastigial nucleus, although the pathways are not clearly known (15-17). Moreover, it remains to be determined if the necessary receptors reside in utricle or semicircular canals. The static deficit in the orthostatic reflex produced by vestibular nerve lesions suggests that at least a part of the deficit can be attributed to gravitational receptors. The fact that stimulation of the vestibular apparatus will evoke the autonomic pattern of orthostasis, however, contrasts with the usual autonomic effect of intensive stimulation of this end organ, namely the autonomic concomitants of motion sickness (19). Possibly, only a very limited part of the input from portions of the vestibular apparatus may be involved in orthostatic control.

A second input to the fastigial nucleus of importance for orthostasis might be from the baroreceptor nerves themselves. Baroreceptor afferents from the carotid sinus nerve project into the paramedian reticular nucleus (20, 21) which, in turn, projects in a reciprocal manner to the fastigial nucleus (22, 23). Electrical stimulation of the fastigial nucleus at the pressor site excites neurons in the paramedian reticular nucleus (24), inhibits the depressor response to baroreceptor stimulation (11, 12, 24), and facilitates the response to carotid artery occlusion. The fact that baroreceptors might trigger the fastigial pressor response, however, seems unlikely, for the responses produced by denervation of baroreceptors combined with lesions of the fastigial nucleus are additive, thereby suggesting that they involve separate pathways. Further support for the distinction between fastigial and baroreceptor mechanisms acting on tilting is provided by the experiments demonstrating a differential sensitivity of the two systems to small doses of barbiturates.

It is evident that, despite partially distinct neural

networks, the baroreceptors act in concert with fastigial and vestibular mechanisms in regulating orthostatic reflexes. Not only do lesions of both inputs impair the orthostatic responses, but the interaction between the fastigial nucleus and the baroreceptor reflexes favors compensatory mechanisms protecting against the hydrodynamic effects of posture by facilitating the pressor responses initiated by withdrawal of baroreceptor stimulation while simultaneously inhibiting the pressor response to baroreceptor stretch (11, 12).

After a combined denervation of baroreceptors and lesions of the fastigial nucleus, the blockade of alpha-receptors by phentolamine produces an even greater impairment of the hypotension resulting from head-up tilting. This finding suggests that some residual reflex activation of sympathetic nerves produced by tilting is preserved in the absence of baroreceptor and vestibular inputs. The pathways mediating this component of orthostatic responses are unknown. It is possible that spinal mechanisms might also participate. The persistence of some orthostatic reflexes in spinal man (25) and the presence of reflex pressor responses to small distortions of the spinal cord transected at the first cervical segment (26) make this possibility viable.

One of the principal deficiencies in the circulatory response to tilting after bilateral lesions of the fastigial nucleus is a delay in the onset of the compensation of the reflex vascular responses to tilting. This fact suggests that the fastigial nucleus response has a relatively shorter latency than does the response of the baroreceptor. This inference is borne out by the studies of Scher and Young (27) and Warner (28) who found that the time for maximal activation of the carotid baroreceptor reflex responses to a square-wave change in blood pressure (27) or electrical stimulation of the carotid sinus nerve (28) was about 15–20 seconds. Based on their calculations the onset time for the reflex vasoconstriction resulting from tilting, if initiated by baroreceptors alone, would be 7–9 seconds, a figure corresponding to the onset of the response in the cats in this study after bilateral lesions of the fastigial nucleus. The vestibular and the fastigial effects on orthostasis, therefore, are preponderant in the very early phases of tilting. The biological utility of these mechanisms in orthostasis is that they provide a short-latency input to the blood vessels. Thus, the first movements of the head during postural changes will excite the vestibular apparatus leading to an initial patterned

activation of preganglionic sympathetic fibers to the heart and the blood vessels. As the movement progresses to a more upright posture, the hydrodynamic changes will excite baroreceptor mechanisms; as these mechanisms begin to participate, they will reinforce and sustain the reflex. The relative contribution of these mechanisms in the alert, unanesthetized cat and their relevance to orthostatic control in man remains to be determined.

Acknowledgment

The authors wish to thank Nancy Shieh for her excellent technical assistance.

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Role of Central and Peripheral Adrenergic Mechanisms in Neurogenic Hypertension Produced by Brainstem Lesions in Rat

By Nobutaka Doba and Donald J. Reis

Role of Central and Peripheral Adrenergic Mechanisms in Neurogenic Hypertension Produced by Brainstem Lesions in Rat

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ABSTRACT

Bilateral lesions of the nucleus tractus solitarius (NTS) in rats result in acute fulminating hypertension (NTS hypertension) as a consequence of central deafferentation of baroreceptors. The hypertension is due to increased peripheral resistance and decreased cardiac output. The hypertension is blocked and cardiac output is increased by phenolamine, trimethaphan (Arfonad), and reserpine but not by propranolol. In the present experiment, systemically administered 6-hydroxydopamine (6-OH-DA) did not alter NTS hypertension if the adrenal glands were intact. Adrenalectomy, however, blocked the lesion-induced rise in blood pressure in 6-OH-DA-treated rats. Intracisternally administered 6-OH-DA (600 μ g) lowered the concentration of norepinephrine only in the spinal cord and blocked the development of NTS hypertension. Local injection of 6-OH-DA into the lateral hypothalamus did not affect the hypertension. Injection of 6-OH-DA into the NTS resulted in a mild, transient elevation in blood pressure. The results of these experiments demonstrate that (1) NTS hypertension is due to increased sympathetic neural discharge, (2) during NTS hypertension sufficient adrenomedullary catecholamines are released to produce hypertension when sympathetic terminals are destroyed, (3) central noradrenergic neurons participate in the expression of NTS hypertension, and (4) baroreceptors can inhibit the release of adrenal catecholamines.

KEY WORDS

brain norepinephrine
nucleus tractus solitarius

blood pressure

6-hydroxydopamine

baroreceptors

adrenal medulla
sympathetic neurons

■ We have recently discovered that in the rat bilateral lesions of the nucleus tractus solitarius (NTS), a nucleus lying dorsolaterally in the medulla oblongata, result in the rapid development of fulminating arterial hypertension (1). It appears that this hypertension (NTS hypertension) is primarily neurogenic and results from central deafferentation of baroreceptors by destruction of their primary synapse within the brain. The consequent release of sympathetic nerve activity then produces a marked increase in peripheral resistance which leads to a reduction in cardiac output, progressive heart failure, pulmonary edema, and death.

Over the past several years increasing evidence has implicated neurons in the brain which synthesize, store, and release the catecholamine

neurotransmitter norepinephrine in the expression of some forms of hypertension (2-8). Recently, Chalmers and Reid (7) have shown that destruction of catecholamine terminals in the central nervous system by intracisternal administration of 6-hydroxydopamine (6-OH-DA) can abort that form of neurogenic hypertension produced by sinoaortic denervation. In the present study we attempted to determine if central catecholamine-producing neurons participate in the expression of hypertension produced by bilateral lesions of the NTS in the rat. We compared the effects of peripheral blockade of sympathetic nerves with the action of 6-OH-DA administered systemically, intracisternally, and intracerebrally.

Methods

The experiments were performed on male Sprague-Dawley rats (300-400 g) anesthetized with 2% halothane in 100% O₂ blown over the nose through a face mask. In acute experiments (1), a polyethylene catheter (PE50, 0.023 inches, i.d.) filled with heparinized saline (20 units/ml) was inserted into the ventral artery of the tail for direct measurement of arterial blood pressure. The catheter was fixed to soft tissue with sutures and connected to a strain-gauge transducer (Statham P23Db) for display on a polygraph (Beckman dynograph recorder 504A). Heart rate was computed

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This research was supported by Grant NS 04876 from the National Institutes of Health and by Grant NGR 33-010-179 from the National Aeronautical and Space Administration.

A preliminary report of this study has been presented at the 3rd International Catecholamine Conference, Strasbourg, May, 1973.

Please address reprint requests to Dr. Donald J. Reis.

Received July 13, 1973. Accepted for publication December 17, 1973.

from the blood pressure pulse wave by a cardiometer (Beckman 9858) and simultaneously displayed. At this time, in selected rats, cannulas or probes for measuring cardiac output or central venous pressure were inserted. In most cases the anesthesia was then discontinued, and the rat was permitted to recover for 30 minutes. Base-line measurements of arterial blood pressure and heart rate were then obtained in the quiet but awake state. The rat was then reanesthetized so that brainstem lesions could be made.

In chronic experiments, systolic blood pressure was measured by a tail cuff method (9) with a systolic auscultatory monitor that suitably amplified Korotkoff's sounds picked up by a small microphone mounted on a tail clip. Measurements were taken from rats placed in a rat holder. Each determination represented the mean of three readings.

Bilateral adrenalectomy was performed through a flank incision while the rats were anesthetized with halothane.

PRODUCTION OF HYPERTENSION BY NTS LESIONS

The methods used to produce NTS lesions have been described previously (1). In brief, the rat was placed in a stereotaxic frame with its head flexed to 45°. The region of the obex was exposed by a limited occipital craniotomy. Under direct visual observation through an operating microscope, a thin monopolar electrode consisting of a stainless steel wire (diameter 0.006 inches) coated with Teflon, bared at the tip for 0.2 mm, and carried in a no. 28 stainless steel hypodermic needle was placed into the region of the NTS by a micromanipulator. This area lies about 0.5 mm lateral to the obex and 0.4 mm beneath the ependymal surface. The lesion was made by passing an anodal d-c current of 5 ma for 1-3 seconds; the cathode was a clip placed in an adjacent muscle. The electrode was removed, and a lesion was then placed at a symmetrical site on the other side of the brainstem.

After surgery the wounds were closed and infiltrated with 2% procaine to minimize pain; the rat was removed from the stereotaxic frame for further observation. When cardiovascular events were monitored, the rat was placed in a small cage through which cannulas or probes were led to appropriate connectors. Cardiovascular activity was measured within 30 minutes after cessation of the anesthesia. At this time the rats were quiet, cardiovascular activity was reasonably stable, and, in rats with NTS lesions, hypertension was well developed. At the termination of the experiment the brain was fixed in 10% formalin for at least 2 weeks; it was subsequently sectioned to confirm the localization of the lesion (1).

MEASUREMENT OF CARDIOVASCULAR ACTIVITY

Cardiac output (CO) was measured by a thermal dilution technique described previously (1). Total peripheral resistance (TPR) was calculated from the formula: $TPR = (P_m - CVP)/CO$, where P_m is mean arterial blood pressure and CVP is central venous pressure measured in the right atrium. Mean arterial blood pressure was derived from the formula: $P_m = (P_s + 2P_d)/3$, where P_s is systolic pressure and P_d is diastolic pressure.

The significance of changes in cardiovascular and other parameters resulting from brainstem lesions was established by a paired *t*-test (10) between postlesion and prelesion measurements. For other data Student's *t*-test for independent samples was applied. A *P* value < 0.05 was considered to be significant.

DRUGS

The following drugs were used in these studies: atropine sulfate (Elkins-Sinn, Inc.), phentolamine (Regitine) (CIBA), propranolol (Sigma Chemical Co.), reserpine sulfate (Serpasil) (CIBA), and trimethaphan camsylate (Arfonad) (Roche). All stock solutions of drugs were diluted in 0.9% w/v NaCl solution before use.

6-HYDROXYDOPAMINE

6-OH-DA hydrobromide (Regis Chemical Co.) was administered systemically by a single intravenous injection of the drug dissolved in physiological saline containing 1 mg/ml of ascorbic acid as an antioxidant. The drug was injected into the femoral vein in rats briefly anesthetized with halothane. Twenty-four hours later the rats either had lesions placed in their NTS or were killed to determine the norepinephrine content of various tissues.

To examine the central effects of the drug, 6-OH-DA was injected intracisternally in rats anesthetized with halothane. The rats were placed in a stereotaxic frame with the head flexed at 45°, and the atlanto-occipital membrane was exposed. The drug was injected into the cisterna magna by a cannula made from 30-gauge stainless steel hypodermic tubing which was, in turn, mounted inside a guide cannula of 25-gauge tubing. 6-OH-DA was dissolved in a physiological salt solution containing ascorbic acid (1 mg/ml) injected over 30 seconds from a 50-μliter Hamilton syringe. The concentration of the drug was adjusted so that for each concentration of 6-OH-DA the injection volume was 10 μliters. To minimize leakage of the drug from the injection site, the cannula was left within the cisterna magna for 15 minutes. Only the ascorbic acid vehicle was administered to the controls.

Intracerebral injection of 6-OH-DA was performed by standard stereotaxic techniques with the rats anesthetized with halothane as previously described (11). A cannula similar to the one described for intracisternal administration of the drug was attached to a 50-μliter Hamilton syringe with a hand dispenser. The injection mixture consisted of 4 μliters of 6-OH-DA (4 μg/μliter as base) dissolved in ascorbic acid (0.8 μg/μliter). Controls received the vehicle alone. For intrahypothalamic administration, the drug was injected bilaterally at a posterior site (A 5.0, RL 2.0, and H 8.0 down) which corresponds to the median forebrain bundle in the lateral hypothalamus. Injection of 6-OH-DA at this site results in profound deficits in behavior and a reduction in norepinephrine and dopamine at and rostral to the site of injection (11). Because of the profound aphagia and adipsia in these rats (11) they were maintained after surgery by tube feeding with a modified diet described by Teitelbaum and Epstein (12). Injections of 6-OH-DA into the NTS were made at the same sites at which the electrolytic lesions produced hypertension.

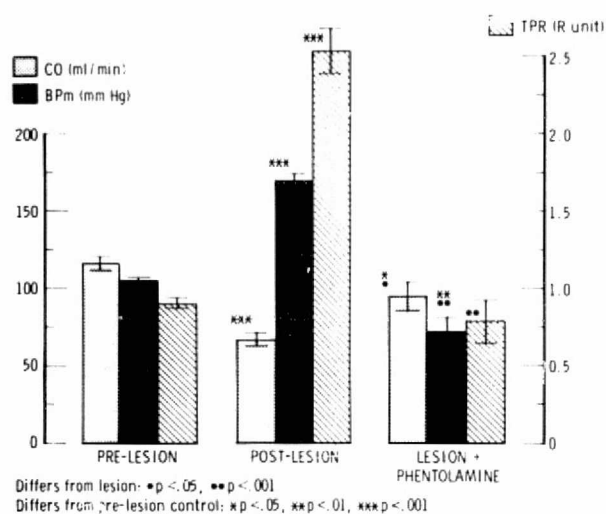


FIGURE 1

Effects of phentolamine on mean blood pressure (BPm), cardiac output (CO), and total peripheral resistance (TPR) in rats with hypertension produced by bilateral lesions of the NTS. Rats were anesthetized with 2% halothane and cannulated; the anesthesia was then discontinued and the prelesion values were obtained 30 minutes later. The rats were reanesthetized, lesions were placed in the NTS, anesthesia was discontinued, and cardiovascular activity was measured 30 minutes later (post-lesion). Phentolamine (1 mg/kg, iv) was then administered and cardiovascular activity was determined within the next 10 minutes. Note the reversal of hypertension, the decrease in total peripheral resistance, and the increase in cardiac output by the drug.

DETERMINATION OF NOREPINEPHRINE CONTENT

The regional concentration of norepinephrine in the central nervous system including the hypothalamus, the lower brainstem (pons and medulla), the cerebellum, and the spinal cord and in the heart and the spleen was measured in either unoperated controls or rats treated systemically or intracisternally with the ascorbic acid vehicle alone or with 6-OH-DA. The amines were assayed by modification of the trihydroxyindole method (13) after extraction on alumina. To evaluate the effects of systemic administration of the drug, the rats were killed by decapitation 24 hours after intravenous administration of 6-OH-DA. The brain and the spinal cord (thoracolumbar segment) were removed and regionally dissected for measurement of norepinephrine. Rats that received 6-OH-DA intracisternally were killed in the same manner 4 days later.

Results

EFFECTS OF PERIPHERAL ADRENERGIC AND GANGLIONIC BLOCKADE ON NTS HYPERTENSION

Both the α -receptor blocking agent phentolamine (1 mg/kg, iv) and the short-acting ganglionic blocking agent trimethaphan (1 mg/kg, iv) immediately reversed toward normal the ar-

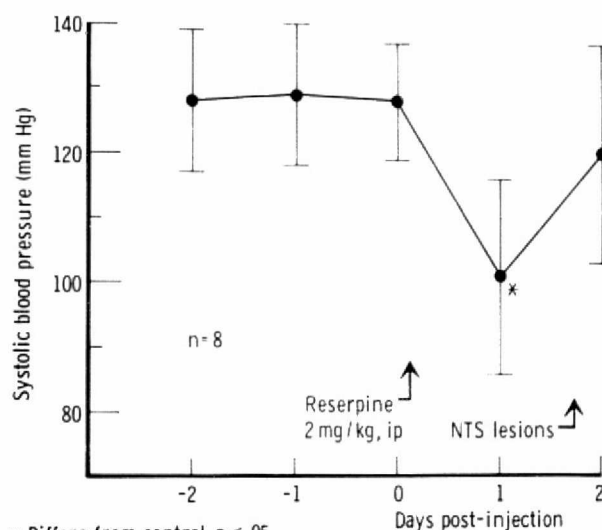


FIGURE 2

Effects of reserpine on arterial hypertension produced by NTS lesions. Systolic blood pressure was measured by a tail cuff method for 3 consecutive days before the injection of reserpine (2 mg/kg, ip). Reserpine produced a significant fall in blood pressure 1 day after injection. Subsequent brain lesions placed in the NTS bilaterally did not elevate blood pressure above control levels.

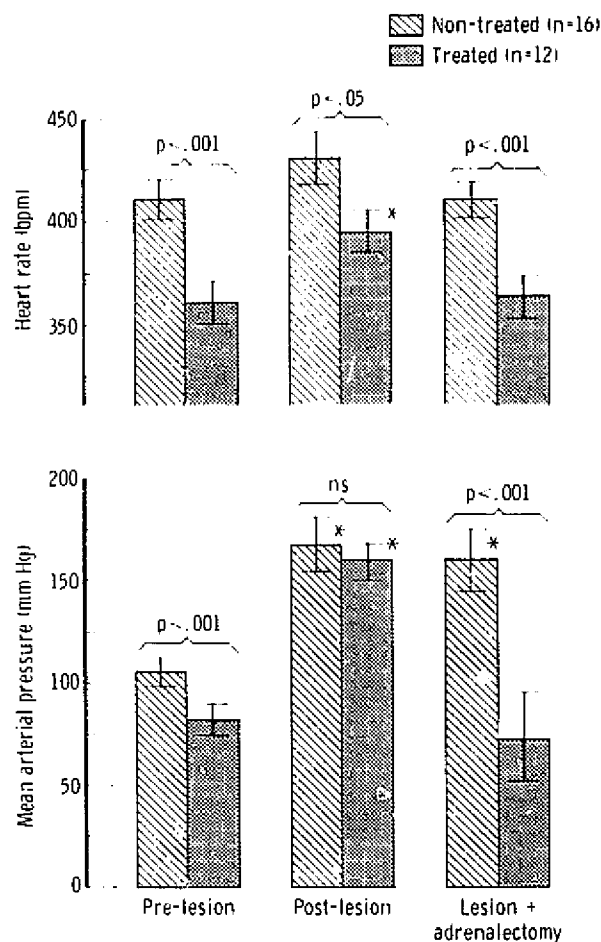
terial hypertension, the elevation in total peripheral resistance, and the decrease in cardiac output associated with the acute hypertension resulting from bilateral lesions of the NTS (Fig. 1). Neither drug produced any change in heart rate.

The β -receptor blocking agent propranolol (1 mg/kg, iv) partially reduced the elevated blood pressure (Table 1). The fall in blood pressure was associated with a significant ($P < 0.01$) fall in heart rate from 368 ± 10 beats/min to 280 ± 15 beats/min; this finding suggests that the decrease in blood pressure produced by propranolol is primarily a consequence of a further decrease in cardiac output. Two of six rats with NTS lesions died with pulmonary edema and acute dilation of the left ventricle shortly after the administration of propranolol; their deaths probably resulted from further compromise of the already impaired cardiac output (Fig. 1).

Development of hypertension after lesion of the NTS could also be prevented by treatment 24 hours before the lesions were established with reserpine (2 mg/kg, ip) (Fig. 2).

EFFECT OF SYSTEMICALLY ADMINISTERED 6-OH-DA ON NTS HYPERTENSION

To determine the role of peripheral noradrenergic neurons in mediating the hyperten-



* Differs from pre-lesion $p < .001$

ns = not significant

FIGURE 3

Effects of 6-OH-DA administered systemically on heart rate (top) and mean arterial blood pressure (bottom) before and after NTS lesions and adrenalectomy. Rats were treated with 6-OH-DA (100 mg/rat, iv). Twenty-four hours later the rats were anesthetized with halothane, and 30 minutes after cessation of anesthesia basal blood pressure and heart rate values were obtained before placement of lesions. Heart rate and blood pressure were directly measured from the tail artery. After measurement of heart rate and blood pressure, the rat was anesthetized with halothane (2%) and lesions were placed in the NTS. Cardiovascular activity was measured 30 minutes later. Note that 6-OH-DA significantly lowered the resting blood pressure and heart rate. Although in both treated and non-treated rats NTS lesions produced the same magnitude of elevation in blood pressure, bilateral adrenalectomy performed 30 minutes after placement of NTS lesions abolished the hypertension in treated rats.

sion produced by bilateral lesions of the NTS, the effect of 6-OH-DA (100 mg/rat, iv) administered 24 hours before surgery was examined. This dose of 6-OH-DA resulted in a significant reduction in the

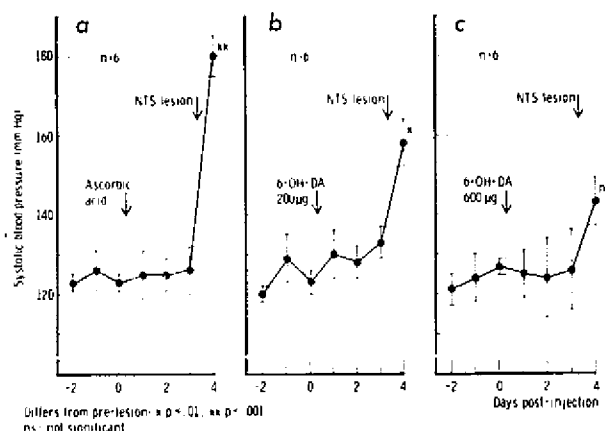


FIGURE 4

Effects of 6-OH-DA administered intracisternally on systolic blood pressure before and after NTS lesions. Blood pressure was measured by a tail cuff method for 3 consecutive days before the intracisternal injection of 6-OH-DA. Control rats (a) were treated with ascorbic acid vehicle alone. Other rats received 200 µg (b) and 600 µg (c) of 6-OH-DA in 10 µliters of ascorbic acid vehicle. Blood pressure was measured for 4 days and then bilateral lesions of the NTS were placed. Note that 6-OH-DA in the higher dose blocked and in the lower dose attenuated the NTS hypertension.

norepinephrine content in the heart and the spleen but not in the brainstem (Table 2).

6-OH-DA administered systemically produced a significant lowering of the resting blood pressure

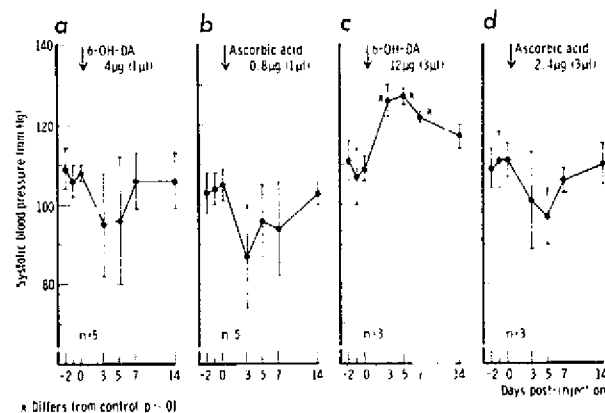


FIGURE 5

Effects on systolic blood pressure of 6-OH-DA directly microinjected into the area of the NTS. Blood pressure was measured by a tail cuff method. Blood pressure was observed for 3 consecutive days before and for 14 days after the microinjection of the drug into the NTS areas. A small dose (a) of 6-OH-DA (4 µg in 1 µliter) did not produce any significant changes in blood pressure. A larger dose (c) of 6-OH-DA (12 µg in 3 µliters) produced a significant rise in blood pressure which gradually returned to control levels after 14 days. Rats treated with either 1 µliter (b) or 3 µliters (d) of ascorbic acid vehicle alone did not show any change in blood pressure.

TABLE 1

Effects of Drugs on Hypertension Produced by Acute Lesions of the NTS in Rats

	N	Mean arterial blood pressure (mm Hg)			P
		Prelesion	Postlesion	Lesion + treatment	
Trimethaphan (1 mg/kg, iv)	6	119 ± 4	158 ± 3	102 ± 4	< 0.05
Propranolol (1 mg/kg, iv)	6	109 ± 4	162 ± 1	145 ± 9	< 0.05

Rats were anesthetized with 2% halothane and cannulated; the anesthesia was then discontinued and blood pressure was measured within 30 minutes to obtain prelesion values. The rats were then reanesthetized, lesions were placed in the NTS, and then anesthesia was discontinued. Blood pressure was measured 30 minutes later and the drugs were administered. The lesion + treatment values were obtained within 10 minutes after the administration of the drug at the time of maximal hypotensive effects. Significance ($P < 0.05$) was established by paired *t*-test comparing prelesion and postlesion blood pressure in individual rats. All values are means ± SE.

and heart rate after 24 hours (Fig. 3); this finding agreed with the observations of de Champlain and van Ameringen (9). Lesions of the NTS in 6-OH-DA-treated rats however, resulted in the development of hypertension similar in magnitude to that in rats not treated with the drug (Fig. 3 bottom). Interestingly, such lesions resulted in a small increase in heart rate in treated rats but not in untreated rats (Fig. 3 top).

The rise in blood pressure resulting from the brainstem lesions in 6-OH-DA-treated rats, but not in the controls, depended on the response of intact adrenal glands. Adrenalectomy in the 6-OH-DA-treated rats (Fig. 3) totally abolished the hypertension. This finding suggests that following the destruction of most peripheral terminals by 6-OH-DA the blood pressure may be partially maintained by the secretion of catecholamines from the adrenal medulla. This finding has been confirmed by the observations of de Champlain and van Ameringen (9). Moreover, it indicates that lesions of the NTS result, in the absence of sympathetic neurons, in a release of adrenal catecholamine sufficient to produce hypertension and possibly to increase heart rate.

EFFECTS OF INTRACISTERNAL INJECTION OF 6-OH-DA ON NTS HYPERTENSION

To determine if central catecholaminergic neurons also participate in the development of the neurogenic hypertension produced by lesions of the NTS, the effects of intracisternal administration of 6-OH-DA were examined. 6-OH-DA was injected intracisternally (600 µg/rat), and blood pressure was measured over the following 3 days. This dose of 6-OH-DA decreased the content of norepinephrine in the spinal cord but not in the hypothalamus, the cerebellum, or the brainstem (Table 3).

6-OH-DA administered intracisternally (600 µg/rat) failed to produce any change in blood pressure (Fig. 4) but did produce a significant bradycardia that was abolished by the administration of atropine (0.2 mg/kg, iv) (Table 4) as noted previously by Chalmers and Reid (7). In contrast to the drug's effects when it is administered systemically, intracisternally administered 6-OH-DA blocked the development of hypertension produced by lesions of the NTS, despite the presence of the adrenal glands. The impairment of the development of hypertension by intracisternally ad-

TABLE 2

Effects of Systemically Administered 6-OH-DA on the Content of Norepinephrine

	Norepinephrine (µg/g wet weight)		% of control	P
	Control	6-OH-DA treated		
Heart	1.175 ± 0.168 (8)	0.210 ± 0.040 (6)	17.9	< 0.001
Spleen	0.949 ± 0.110 (7)	0.358 ± 0.037 (7)	37.8	< 0.001
Brainstem	0.830 ± 0.078 (7)	0.989 ± 0.105 (7)	119	NS

6-OH-DA (100 mg/rat, iv) was administered in 0.5 ml of ascorbic acid vehicle. Twenty-four hours later the rats were killed, and the tissues were removed and assayed for norepinephrine. The number of rats tested is given in parentheses. NS = not significant.

TABLE 3

Effects of 6-OH-DA Administered Intracisternally on the Norepinephrine Content of Selected Brain Regions and the Heart

	Norepinephrine ($\mu\text{g/g}$ wet weight)		
	Control	Ascorbic acid	6-OH-DA
Hypothalamus	2.578 ± 0.109 (3)	2.428 ± 0.416 (3)	2.827 ± 0.507 (4)
Cerebellum	0.522 ± 0.096 (7)	0.640 ± 0.096 (6)	0.595 ± 0.039 (7)
Brainstem	0.830 ± 0.078 (7)	0.994 ± 0.092 (7)	0.933 ± 0.083 (6)
Spinal cord	0.942 ± 0.105 (7)	0.760 ± 0.040 (7)	$0.499 \pm 0.070^*$ (7)
Heart	1.175 ± 0.168 (8)	1.228 ± 0.150 (7)	1.149 ± 0.077 (7)

6-OH-DA (600 μg) in 10 μliters of ascorbic acid vehicle or vehicle alone were administered intracisternally. Four days later the rats were killed, and the tissues were removed for estimation of norepinephrine. Controls were killed by cervical dislocation. The number of rats studied is given in parentheses.

*Differs from control, $P < 0.01$.

ministered 6-OH-DA was dose related. Treatment with 200 $\mu\text{g}/\text{rat}$ of 6-OH-DA failed to abolish and only partially blocked the magnitude of the lesion-induced hypertension (Fig. 4).

EFFECTS OF INTRACEREBRAL INJECTION OF 6-OH-DA ON NTS HYPERTENSION

We have previously demonstrated that the development of NTS hypertension depends on the integrity of structures lying above the midbrain (1). Midcollicular decerebration prevents the development of hypertension evoked by NTS lesions or reverses the hypertension once it is established. To determine if 6-OH-DA acts to block the development of NTS hypertension by destroying catecholamine axons terminating in or passing through the hypothalamus, the drug was directly injected into the lateral hypothalamus. Since injections produce profound behavioral deficits and a significant fall in norepinephrine content in the hypothalamus and the forebrain and in dopamine content in the cau-

date nucleus (11).

Injection of 6-OH-DA into the lateral hypothalamus resulted in the development of the lateral hypothalamic syndrome of aphagia and adipsia and failed to produce any effects on blood pressure or heart rate of the control rat. It also failed to block the elevation in blood pressure produced by lesions of the NTS.

6-OH-DA (4 $\mu\text{g}/\mu\text{liter}$) was also microinjected bilaterally into the NTS. Such injections resulted 3 days later in a slight but statistically significant elevation in blood pressure which gradually returned to control levels by the fourteenth day (Fig. 5c). The effect of such an injection was not due to mechanical destruction of the tissue within the NTS since an isovolumetric injection of ascorbic acid failed to produce the effect (Fig. 5d). A smaller dose of 6-OH-DA injected into the NTS did not produce any changes in blood pressure (Fig. 5a and b).

TABLE 4

Changes in Heart Rate after Intracisternal Injection of 6-OH-DA

	N	Heart rate (beats/min)
Ascorbic acid	6	365 ± 13
6-OH-DA (200 μg)	6	376 ± 20
6-OH-DA (600 μg)	6	$280 \pm 12^*$
6-OH-DA (600 μg) + atropine	6	$390 \pm 22^\dagger$

*Differs from heart rate after administration of ascorbic acid, $P < 0.001$.

†Differs from heart rate after administration of 600 μg of 6-OH-DA, $P < 0.001$.

Discussion

The present study demonstrated again that bilateral lesions of the NTS in the rat, which centrally deafferentate baroreceptors (1, 14, 15), result in the development of a marked arterial hypertension in association with increased total peripheral resistance and decreased cardiac output. The finding that the hypertension, the increased resistance, and the decreased cardiac output can be reversed by α -receptor or ganglionic blockade further supports our views that (a) the hypertension is neurogenic and due to a marked augmentation of sympathetic nerve activity, (b) the hypertension is exclusively the result of changes in peripheral resistance, and (c) the fall in cardiac output is primarily a consequence of left ventricular overload.

Therefore, systemically administered 6-OH-DA, which destroys sympathetic nerve terminals in the heart and the spleen (9, 16, 17) and partially in the larger arteries (18) and which functionally impairs the vasoconstriction elicited by stimulation of sympathetic nerves (19, 20), should block the elevation in blood pressure produced by bilateral lesions of the NTS. (Although 6-OH-DA depletes 80–90% of the stores of norepinephrine in the heart and the spleen, Berkowitz et al. [18] have shown that comparable doses of the drug only reduce the concentrations of the amine in the aorta and the mesenteric artery of the rat to about 60% and 30%, respectively. However, although 6-OH-DA produces only partial chemical denervation of the larger vessels, the functional effects on systemic vasoconstriction may be almost complete [19, 20]. This functional effect might result because 6-OH-DA more successfully denervates smaller arterioles such as those in the spleen [21], thereby blocking vasoconstriction in the principal resistance segment of the vascular tree.) Systemically administered 6-OH-DA, however, produced a marked lowering of resting blood pressure and heart rate but did not impair the rise in blood pressure produced by NTS lesions when the adrenal glands were not removed. However, the fact that subsequent acute adrenalectomy blocked the lesion-induced elevation in blood pressure raises several interesting points. First, it adds to other evidence that 6-OH-DA administered systemically does not significantly destroy the chromaffin cells of the adrenal medulla (22, 23) and thus leaves the secretion of adrenomedullary catecholamines relatively intact. Second, this finding further supports the view of de Champlain and van Ameringen (9) that in the ab-

sence of sympathetic nerve endings, the secretion of catecholamines from the adrenal medulla is sufficient to maintain vascular tone near normal. Third, the release of catecholamines from the adrenal medulla after NTS lesions is sufficient to elevate the blood pressure to a degree comparable to that which occurs with sympathetic nerves intact.

However, since adrenalectomy in rats with intact sympathetic neurons does not attenuate the rise in blood pressure evoked by brainstem lesions (1), the present experiments suggest that 6-OH-DA-treated rats secrete more adrenomedullary catecholamines in response to NTS lesions than do rats not treated with the drug or that after treatment with 6-OH-DA adrenal catecholamines exert a more powerful action on end organs. It is not possible without direct measurement of circulating catecholamines to exclude augmented release. The latter mechanism is probable, however, because the destruction of sympathetic nerve terminals by 6-OH-DA results in a form of denervation supersensitivity as a consequence of impairment of a presynaptic form of physiological inactivation of the amines by reuptake and sequestration into storage vesicles (24, 25). The activation of the adrenal medulla in NTS hypertension parallels the increase in adrenal medullary activity that occurs in hypertension produced by treatment with deoxycorticosterone acetate (DOCA) and salt (9).

Finally, since the effect of NTS lesions is to deafferentate baroreceptors centrally (1, 14, 15), our findings suggest that baroreceptors might exert a tonic inhibition on adrenal medullary secretion. This possibility is reinforced by the observations of De Quattro et al. (26) that deafferentation of sinoaortic nerves results in an increased neurogenic drive to the adrenal medulla and also by the longstanding observations that carotid occlusion results in augmented reflex release of the adrenal catecholamines (27–30).

Our finding that 6-OH-DA administered intracisternally abolishes the lesion-induced elevation in blood pressure demonstrates that central catecholamine-producing neurons participate in the expression of NTS hypertension. The effect of 6-OH-DA on NTS hypertension is clearly through central and not peripheral mechanisms. This conclusion is supported by our findings that intracisternally, in contrast to systemically, administered 6-OH-DA (a) reduced norepinephrine within the central nervous system but not in the heart, (b) did not alter the resting blood pressure, and (c) abolished the hypertension produced by NTS le-

sions despite the presence of the adrenal glands. It is probable that central noradrenergic rather than dopaminergic neurons are necessary for the full expression of the hypertension since intra-hypothalamic injections of 6-OH-DA, which damage the principal dopaminergic projections of brain (11), fail to impair the hypertension.

It is unlikely that the noradrenergic projections in the hypothalamus or the forebrain are critical in maintaining NTS hypertension, since 6-OH-DA-induced lesions effectively destroy most noradrenergic terminals within these regions (11). It is more likely, as Chalmers and Reid (7) have suggested, that a bulbospinal noradrenergic system is critical, since our injection of 6-OH-DA resulted in a significant fall in norepinephrine only in the spinal cord. The fact that intracisternally administered 6-OH-DA attenuated hypertension induced by NTS lesions further supports the studies of Chalmers and Reid (7) on neurogenic hypertension produced by sinoaortic denervation in the rabbit. These findings suggest a common engagement of central noradrenergic neurons, probably bulbospinal, in neurogenic and possibly other forms of experimental hypertension.

On the other hand, our observation that local injection of 6-OH-DA into the NTS produces a transient hypertension indicates that not all noradrenergic systems facilitate arterial blood pressure. Indeed it suggests that some systems may serve to depress arterial blood pressure. The NTS and the adjacent medial-dorsal regions of the medulla in the rat are richly innervated with noradrenergic terminals and also contain some cell bodies of noradrenergic neurons (31, 32). The role of the noradrenergic innervation of the NTS is unknown. However, recent studies (33, 34) on the pharmacological action of the centrally acting hypotensive agent londine, a drug that acts as an α -receptor agonist, have suggested that norepinephrine may produce its hypotensive actions by activation of baroreceptor pathways (35, 36) possibly within the NTS (36). Thus, it is conceivable that the single neurotransmitter norepinephrine may have opposing central actions on arterial blood pressure depending on the site at which it is released, the origin of the parent cell body, and, possibly, the nature of the receptor. Our findings suggest that in the NTS norepinephrine opposes a rise in blood pressure, but in the spinal cord it facilitates a rise in blood pressure.

It is unlikely that the whole syndrome of NTS hypertension can be explained exclusively on the

basis of the destruction of noradrenergic terminals in the NTS for two reasons. First, in contrast to NTS hypertension, the effect produced by microinjection of 6-OH-DA into the NTS is mild and transient. Second, NTS hypertension is similar to that produced by selective denervation of baroreceptors (1), yet baroreceptor afferents are not noradrenergic. It is more probable that the noradrenergic innervation of the NTS primarily modulates baroreceptor reflex mechanisms rather than serving as the primary activator of the reflex.

Acknowledgment

The authors wish to thank Jana Iglich for excellent technical support.

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The Central Nervous System and Neurogenic Hypertension

Donald J. Reis and Nobutaka Doba

IT HAS LONG been proposed that the sympathetic nervous system may play a critical role in initiating and/or sustaining the disease of essential hypertension.¹⁻⁴ The long-standing knowledge that drugs that block sympathetic neurotransmission peripherally reduce the elevated blood pressure in patients with hypertension⁴ and recent evidence, acquired by application of new and sensitive methods, that patients with essential hypertension have elevated levels of circulating catecholamines^{5,6} have added support to the hypothesis.

The precise manner in which the sympathetic neuron participates in hypertension remains to be elucidated. Conceivably, dysfunction of the machinery in the sympathetic terminal itself could lead to inadequate inactivation of neurotransmitters.⁷ A second mechanism could be a primary alteration in vascular smooth muscle, which might lead to heightened reactivity or structural changes in the effector organ leading to increased vascular resistance and elevation of blood pressure.^{8,9} In this instance, the sympathetic neuron would play a permissive role in the development of hypertension. Finally, a defect within the reflexive pathways that regulate the magnitude of the sympathetic discharge by the brain, could be at fault, thereby leading to undampened activity of the sympathetic neurons. Such increased sympathetic activity might lead to transient and reversible hypertension but conceivably could lead to secondary changes within blood vessels that might fix the hypertensive state.^{2,8,9} Such defects could be located within peripheral receptors, or within the areas of brain necessary for integrating the reflexes into centrally patterned changes in circulatory function.

It is widely accepted that the central nervous

system (CNS) plays a critical role in governing the behavior of the circulation.^{10,11} The functions of the CNS in circulatory control are many. Among the more important are the following. First, within the confines of the CNS, the cell bodies of preganglionic sympathetic and parasympathetic neurons reside. These cells directly influence the performance of virtually every segment of the vascular tree and every dimension of cardiac performance. Second, the CNS receives information of the second-to-second performance of the circulatory system. Such information is obtained through the highly specialized cardiopulmonary receptors and also, probably, from other sensory afferents. Within the brain, this information is integrated into appropriate reflex patterns serving to maintain relatively steady-state conditions (e.g., by baroreceptor reflexes) within the circulatory system or to reset the circulatory system preparatory to the induction of a behavioral response (e.g., the hypertensive response to noxious stimulation of the limbs). Third, neurons within the CNS exert tonic excitatory and probably inhibitory control over the discharge of autonomic nerves and are necessary for continuous maintenance of the blood pressure within normal limits. Fourth, the brain serves to match appropriate circulatory patterns to widely different behaviors, such as feeding, sleeping, diving, or attack.^{10,11} Indeed, the coupling of circulatory changes to behavior appears to be one of the principal functions of the circulatory representation within the brain. Finally, the CNS exerts important indirect regulation of the circulation by its role in controlling the fluid, endocrine, and electrolyte balance within the circulation, largely regulated by the hypothalamo-pituitary axis.

Such extensive control systems of the brain are naturally highly complex. They are subserved by extensive and precisely demarcated neural networks whose identity has only been partially elucidated. Moreover, within these networks, specific neurotransmitters function to communicate the information coded into discharge patterns within specific pathways. These chemical mediators and the enzymes subserving their synthesis

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and degradation are important control sites on which drugs may act to influence the circulation. Indeed, there is increasing evidence that a number of agents commonly used in the clinical treatment of hypertension may act through the brain.

In this essay, we shall focus on the experimental evidence that suggests that manipulation of some of the central neural mechanisms regulating the outflow of the autonomic nervous system and/or the major afferent inputs into these systems can lead to arterial hypertension. Most of the material that will be reviewed will be drawn from experiments with animals. For the purposes of this article we will consider *neurogenic hypertension* as arterial hypertension that is initiated and/or sustained by sympathetic neural activity resulting mainly from manipulation of the primary reflex arcs governing the circulation and/or central integrating mechanisms for these reflexes.

BARORECEPTORS AND HYPERTENSION

Baroreceptor Reflexes

Peripheral mechanisms. The baroreceptor cardiovascular reflexes consist of a sequence of circulatory adjustments in response to changes in the discharge of stretch receptors lying within specialized areas of the arterial bed.^{12,13} The areas in which these receptors are most densely concentrated lie within the carotid sinus and aortic arch. The afferent fibers from the former, are conducted to the brain through the carotid sinus branch of the glossopharyngeal nerve, while impulses from the latter traverse the aortic depressor nerve, a branch of the vagus. Baroreceptor reflexes can be evoked from other segments of the arterial bed of the chest including the common carotid and subclavian arteries. Afferent fibers from these sites presumably ascend in the main trunk of the vagus.¹² Since the baroreceptors are tonically active under conditions of normal arterial pressure^{12,13} they can signal an increase and decrease in stretch in the vessel wall by increasing or decreasing their discharge frequency.

The cardiovascular responses to alterations in baroreceptor activity traditionally have received the greatest attention. The reflex responses to an increase in baroreceptor discharge produced by static or phasic stretch of the receptors consists of a widespread inhibition of the discharge of pre-ganglionic sympathetic neurons and excitation of the cardiac vagus.¹²⁻¹⁶ The consequence of such

sympathetic inhibition primarily consists of a decrease in background vasoconstriction, which is greatest in those vascular beds with the highest tonic sympathetic activity.¹⁴ Thus, there is a reduction of total peripheral resistance and, as a consequence of bradycardia and diminished venous return, a decrease in the cardiac output. The net result is a fall of the systemic blood pressure. A depressor response similar to that evoked by stretch of systemic baroreceptors also can be evoked from cardiac mechanoreceptors^{13,15,16} probably engaging in similar central pathways.

The reflex circulatory responses to a decrease in baroreceptor activity is opposite in direction to that produced by stretch of the receptors.^{12,13} Thus, decreasing the discharge of carotid sinus baroreceptors by immobilization of the carotid sinus in a plaster cast,¹⁷ by clamping the carotid artery proximal to the sinus, or by transecting the carotid sinus nerve,¹² results in an increase in sympathetic neural activity in some but not all projection fields (a differentiated response), an increase in total peripheral resistance and heart rate (probably without change in the cardiac output), and an elevation of the systemic blood pressure. Adrenomedullary catecholamines are also probably released.¹³

In addition to fluctuation of stretch of the vessels imposed by the arterial pulse, the activity of vascular baroreceptors may also be altered by changes from within the end organ. For example, the capacity to modify baroreceptor reflexes at the receptor level has been amply demonstrated by pharmacologic studies in which drugs directly applied to the carotid sinus have been shown to result in powerful activation of baroreceptor reflexes.^{12,15,16} Most of these drugs are vasoconstrictors, and it is generally believed that these responses are a consequence of contraction of smooth muscle near the sinus, thereby indirectly stimulating the receptors. However, since there is also a powerful local innervation of the carotid sinus by sympathetic fibers,¹⁸ it has been postulated that some control of the carotid sinus reflex can be exerted through local sympathetic discharge.^{18,19} Indeed, the sympathetic innervation of baroreceptors may represent an important control mechanism for the blood pressure and may be under control by the brain. Changes in the baroreceptors have been postulated as possibly playing a role in resetting baroreceptor reflexes in

hypertension²⁰⁻²² and have been shown to change sensitivity in some hypertensive states.²⁰⁻²²

The baroreceptors have long been considered an important control system in regulating arterial pressure, peripheral resistance, and the distribution of blood flow. They serve, through negative feedback, to maintain cardiovascular conditions within a specific range, which is characteristic for different species.^{12,13,22} Baroreceptors have long been viewed as being important in buffering the circulation from abrupt elevations of blood pressure. Perhaps a more important function is the protection that baroreceptors offer against a fall of blood pressure, particularly in response to assumption of an upright posture. This latter function is probably one of the major determinants in initiating and sustaining orthostatic reflexes.²³

While the principal emphasis on the baroreceptors has focussed on their roles in the neurogenic regulation of the circulation, it should be emphasized that the discharge of baroreceptors influences other physiologic functions as well. Thus, baroreceptors of the carotid sinus have been shown to play an important role in the maintenance of fluid balance by regulating the output of antidiuretic hormone,^{24,25} in regulating the output of anterior pituitary hormones,²⁶ and in modulating sleep and wakefulness cycles.^{27,28}

Central neural integration of baroreceptor reflexes. The nature of the central neural mechanism governing baroreceptor reflexes has only been elaborated in recent years. The baroreceptor afferents from the carotid sinus and probably aortic arch enter the brain stem through the roots of cranial nerves IX and X and descend along the tractus solitarius to synapse in part within the bed nucleus of the solitary tract, the *nucleus tractus solitarii* (NTS)²⁹⁻³⁷ (Fig. 1). The principal evidence is based on electrophysiologic observation using techniques of antidromic stimulation of primary afferent fibers of the CNS,³¹ the orthodromic evocation of evoked field and unit responses recorded extracellularly and intracellularly,^{30,32-34,36} and identification of spontaneously active units of the brain stem discharging in synchrony with the pulse and whose activity is abolished by carotid occlusion.³⁷ An example of the electrophysiologic characteristics of a carotid sinus neuron in the brain is seen in Fig. 2.

In addition to the heavy innervation of the NTS by baroreceptors (and chemoreceptors) there is, at

least in the cat, a direct projection of carotid sinus afferents into specific nuclear regions of the bulbar reticular formation, primarily within the confines of paramedian reticular nucleus.^{31,32,36,37} Some fibers possibly terminate in more dorsally situated areas including the parahypoglossal area.³⁵ From the NTS and the paramedian reticular nucleus activity evoked from the carotid sinus region projects into other nuclei of the reticular formation both at the same segmental level and also through ascending pathways rising through the dorsolateral pontine reticular formation.³² The projections of the carotid sinus into the cat's brain stem are summarized in Fig. 1. The trajectory of the ascending pathway above the pons carrying information from baroreceptors is not known. However, suprapontine and presumably hypothalamic regions appear to be of importance in mediating the pressor response to withdrawal to baroreceptor activity^{38,39} as well as the effects of the baroreceptors on regulating the secretion of posterior pituitary hormones.^{24,25} The intimate connections within the medulla, through which baroreceptor afferents ultimately modulate descending projections to spinal preganglionic neurons, have only partially been identified.^{40,41}

The baroreceptor reflexes within the brain stem are organized in a complex manner and are under a considerable degree of suprasegmental control that is both tonic and phasic.⁴²⁻⁴⁹ The excitability of the pressor, depressor, and cardiovagal components can be altered by electrical stimulation or lesions of widely divergent areas of the brain, including cerebellum, hypothalamus, and limbic systems. Moreover, baroreceptor reflex excitability can be demonstrated to be modified independently of any changes in the systemic blood pressure⁴³ indicating that the neuronal substrate of the reflex system is in part distinct from that system that maintains normal blood pressure levels.^{37,43}

Experimental Neurogenic Hypertension Produced by Reduction of Baroreceptor Activity in the Periphery

Hypertension with sinoaortic immobilization. Since the baroreceptors of the carotid sinus region are stretch receptors, attempts have been made to inactivate them by encasing the region of the carotid sinus in rigid casts. This presumably interferes with arterial stretch in response to the

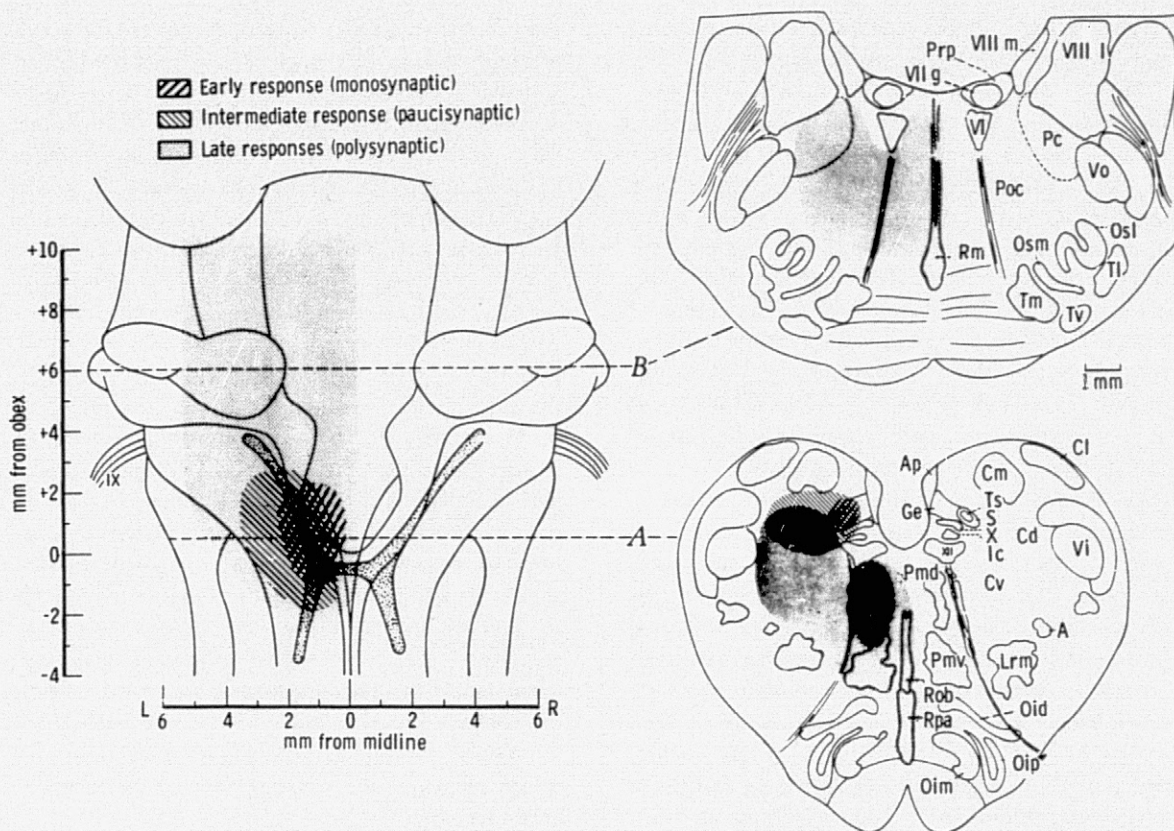


Fig. 1. (Left) Summary of distribution of early (monosynaptic), intermediate (disynaptic), and late (polysynaptic) responses evoked by electric stimulation of carotid sinus nerve in the cat in medulla and pons projected onto floor of fourth ventricle. Stippled bilateral structure represents nucleus tractus solitarius. (Right) Summary of nuclear distribution of early (monosynaptic), intermediate, and late responses evoked by electric stimulation of carotid sinus nerve in the cat in representative sections of medulla (A) and pons (B). Rostrocaudal level of each transection is as indicated. Abbreviations: A, nucleus ambiguus; Ap, area postrema; Cd, nucleus medullae oblongatae centralis, subnucleus dorsalis; Cl, nucleus cuneatus lateralis; Cm, nucleus cuneatus medialis; Cv, nucleus medullae oblongatae centralis, subnucleus centralis; Gc, nucleus gigantocellularis; Ge, nucleus tractus solitarius, subnucleus gelatinosus; Lrm, nucleus lateralis reticularis, subnucleus magnocellularis; Oid, nucleus olivaris inferior accessorius dorsalis; Oim, nucleus olivaris inferior accessorius medialis; Oip, nucleus olivaris inferior principalis; Osl, nucleus olivaris superior lateralis; Osm, nucleus olivaris superior medialis; Pc, nucleus parvocellularis; Pmd, nucleus paramedianus reticularis, subnucleus dorsalis; Pmv, nucleus paramedianus reticularis, subnucleus ventralis; Poc, nucleus pontis centralis caudalis; Prp, nucleus praepositus hypoglossi; Pyr, tractus pyramidalis; Rm, nucleus raphae magnus; Rpa, nucleus raphe pallidus; S, Nucleus tractus solitarius; Tl, nucleus trapezoidalis lateralis; Tm, nucleus trapezoidalis medialis; Tv, nucleus trapezoidalis ventralis; V, nucleus nervi trigemini; Vi, nucleus nervi abducentis; VII, nucleus nervi facialis; VIIg, genu nucleus nervi facialis; VIII, nucleus vestibularis lateralis; VIII m, nucleus vestibularis medialis; X, nucleus nervi vagi dorsalis motorius; and XII, nucleus nervi hypoglossi. (By permission.³²)

arterial pulse wave and reduces the afferent barrage of carotid sinus baroreceptors.^{17,50} In this manner, Burstyn et al.,¹⁷ have recently produced chronic arterial hypertension in rabbits by the placement of rigid casts around the carotid sinus regions bilaterally. They thoughtfully comment on the fact that despite the integrity of other baroreceptor beds, notably in the aortic arch, hypertension develops after immobilization of the carotid sinus region. This is in contrast to the situation of selective denervation of carotid sinus

nerves, which, in the presence of intact aortic nerves, will not produce sustained hypertension. It is possible that in some manner the disruption of the patterned impulse activity arising from a sequenced input of various baroreceptors or perhaps the preservation of chemoreceptor input permits the hypertension to develop.

Hypertension with sinoaortic denervation. The discovery^{2,12,51} that bilateral denervation of baroreceptors of the carotid sinus and aortic arch in the rabbit by transection of carotid sinus and aortic

likely that a rather widespread activation of sympathetic vasomotor fibers occurs after sinoaortic denervation, since reversal of the hypertension can only be accomplished by total sympathectomy.⁵⁸

Hypertension with chronic carotid occlusion.

Occlusion of the carotid arteries bilaterally can transiently elevate the arterial pressure by reducing stretch of carotid sinus baroreceptors.^{12,13} Chronic ligation of the carotid arteries has been claimed to elevate the blood pressure chronically.^{2,67} However, it is not certain whether or not chronic occlusion may not produce its effects by cerebral ischemia, as will be discussed below.

Neurogenic Hypertension Resulting From Central Deafferentation of Baroreceptors: A NTS Hypertension in the Rat

The recent discovery of the central projections of baroreceptor afferents²⁹⁻³⁷ and the discovery by Miura and Reis³⁷ that small bilateral lesions of one of the primary terminal sites of baroreceptors, the region of the nucleus tractus solitarius (NTS) at the level of the obex, will abolish all baroreceptor reflexes (Fig. 3) led us to examine^{68,69} whether or not neurogenic hypertension could

be produced in the rat by central deafferentation of baroreceptors. This study was performed on rats in whom intravascular cannulae were inserted while the animal was briefly anesthetized with halothane. The anesthesia was discontinued while basal cardiovascular activity was determined, the animal was then reanesthetized with halothane and small bilateral lesions placed in the NTS at the obex. The animal was allowed to recover from anesthesia, and cardiovascular function was observed.

Small bilateral electrolytic lesions of the dorsal brain stem that destroyed the NTS at the level of the obex (Fig. 4) invariably result in arterial hypertension. The hypertension appears within 5 min after the halothane anesthesia is discontinued, and by 30 min the hypertension is stable and sustained until the onset of heart failure (Fig. 5). By 30 min after termination of anesthesia, these rats show an increase in the systolic, diastolic, and pulse pressures unassociated with any change in heart rate. Systolic pressure rises from a mean of 125 at control levels to over 200 mm Hg after the lesion. The elevation of blood pressure is entirely attributable to an increase in the total peripheral resistance (of the order of 250%). The

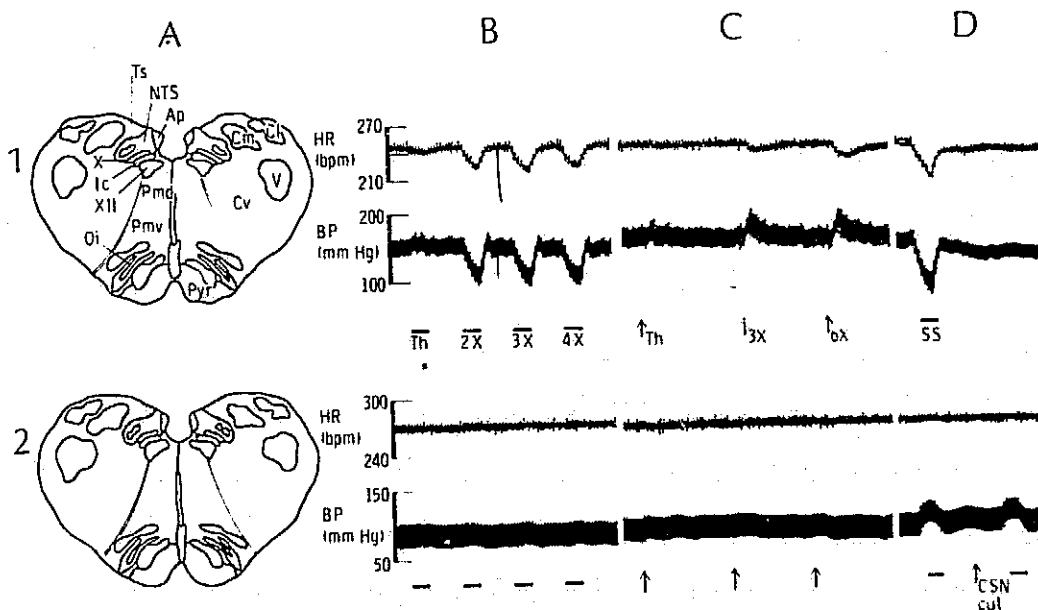


Fig. 3. Effects of bilateral lesions of the nucleus of the solitary tract (NTS) on reflex blood pressure responses to electrical stimulation (—) of left carotid sinus nerve (column B) with: threshold stimulus current (Th); at multiples of the threshold (X); with closed intraarterial injection of lobeline (column C); at the threshold dose (↑Th); at multiples of the threshold dose (↑X); with sinus stretch (SS) (column D). Row 1 is before, and row 2 30 min after the bilateral lesions of NTS indicated in column A. Note that after NTS lesions sinus stretch results in a rise instead of fall of blood pressure, which is unaffected by transection of the carotid sinus nerve. (By permission.³⁷)

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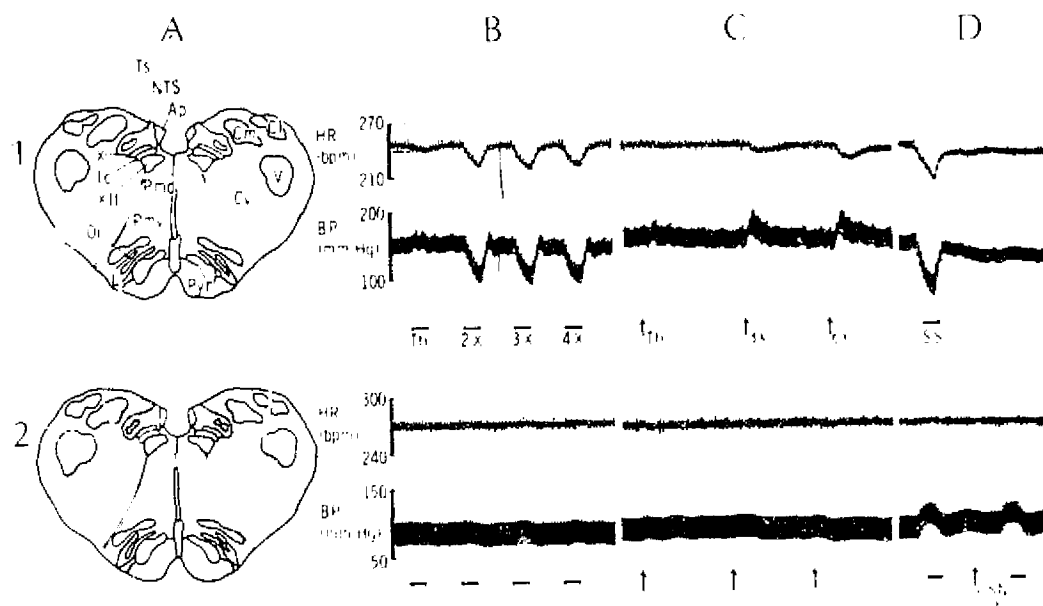
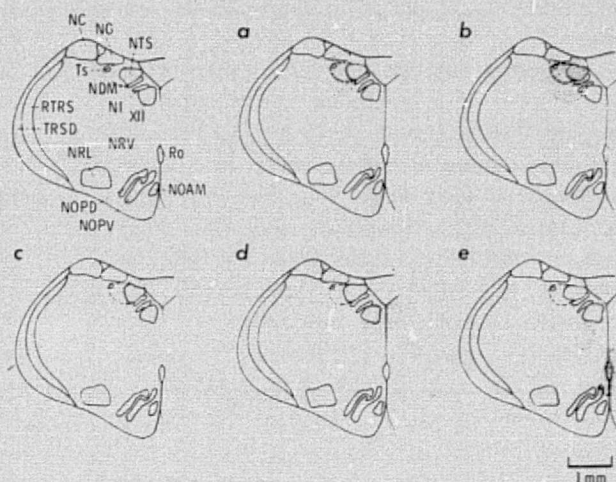


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Fig. 4. Representative brain-stem lesions effective or ineffective in producing neurogenic hypertension in the rat. The lesions are projected on a cross section of the medulla of the rat at the level of the rostral third of the inferior olivary nucleus. Only one side of generally symmetrical lesions is shown. (A and B) Lesions were associated with hypertension. (C, D, and E) The lesions failed to produce hypertension. Abbreviations are according to Cragie. NC, nucleus cuneatus; NDM, dorsal motor nucleus of the vagus; NG, nucleus gracilis; NI, nucleus intercalatus; NOAM, nucleus olivaris accessorius; NOPD, nucleus olivaris principalis (pars dorsalis); NOPV, nucleus olivaris principalis (pars ventralis); NRL, nucleus reticularis lateralis; NRV, Subnucleus reticularis ventralis medullae oblongatae; NTS, nucleus tractus solitarius; Ro, nucleus raphe obscurus; RTRS, radix tractus spinalis nucleus trigemini; TRSD, tractus spinocerebellaris dorsalis; Ts, tractus solitarius; and XII, nucleus hypoglossi. (By permission.⁶⁸)



elevated resistance produces an overload of the left ventricle, a consequent reduction of stroke volume, and an increase in end-diastolic pressure resulting in a fall of cardiac output to 60% of normal. The elevated peripheral resistance ultimately leads to left ventricular failure, and by 4-6 hr after placement of the lesions the animals rapidly develop acute pulmonary edema and die.

The hypertension produced by NTS lesions is almost certainly neurogenic and is due to a dif-

ferentiated activation of sympathetic neurons to blood vessels, since there is no evidence of widespread sympathetic activation (e.g., no pupillary dilatation or piloerection). It is not attributable to changes in blood gases nor to the release of pressor substances from the kidneys or the adrenal glands, since prior adrenalectomy does not reduce the magnitude of the hypertension. On the other hand, ganglionic blocking agents or the α -adren-ergic blocking agent, phentolamine, will reduce

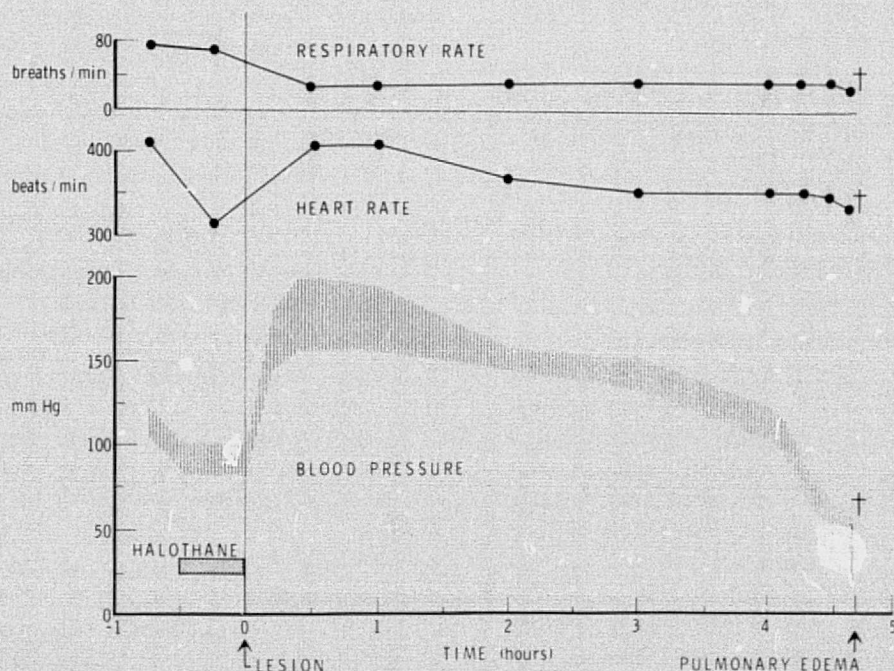


Fig. 5. Time course of changes in systemic arterial blood pressure, heart rate, and respiratory rate in a representative unanesthetized rat after production of bilateral lesions in the NTS. Just prior to death the rat developed pulmonary edema. (By permission.⁶⁸)

the elevated blood pressure and reverse the cardiac failure.⁶⁹

All evidence suggests that NTS lesions produce their release of sympathetic neural activity by damaging the central projections of baroreceptors. It is more intense than the hypertension produced by sinoaortic denervation alone probably because the central lesion interrupts depressor reflexes arriving from the cardiopulmonary mechanoreceptors that traverse the vagus nerve. It is extremely unlikely to be the consequence of any irritative (i.e., stimulatory) effect of the lesion.⁶⁸

One of the most characteristic features of the hypertension evoked by NTS lesions is its dependence on the integrity of structures lying above the midbrain. Midcollicular decerebration will abort the development of hypertension before NTS lesions are placed or abolish the hypertension once the lesions are established. The importance of rostral regions of the brain in mediating the hypertension parallels the observations^{38,39} that the reflex hypertension elicited by sinoaortic denervation or carotid occlusion is abolished by decerebration. The findings suggest that baroreceptors after terminating in the medulla are engaged in long loop cardiovascular reflexes with higher brain areas. The precise localization of the rostrally situated regions necessary for this mode of hypertension remains to be established.

NEUROGENIC HYPERTENSION WITH INCREASED INTRACRANIAL PRESSURE AND DISTORTION OF THE BRAIN STEM

Arterial Hypertension Produced by Acute Elevation of Intracranial Pressure: The Cushing Response

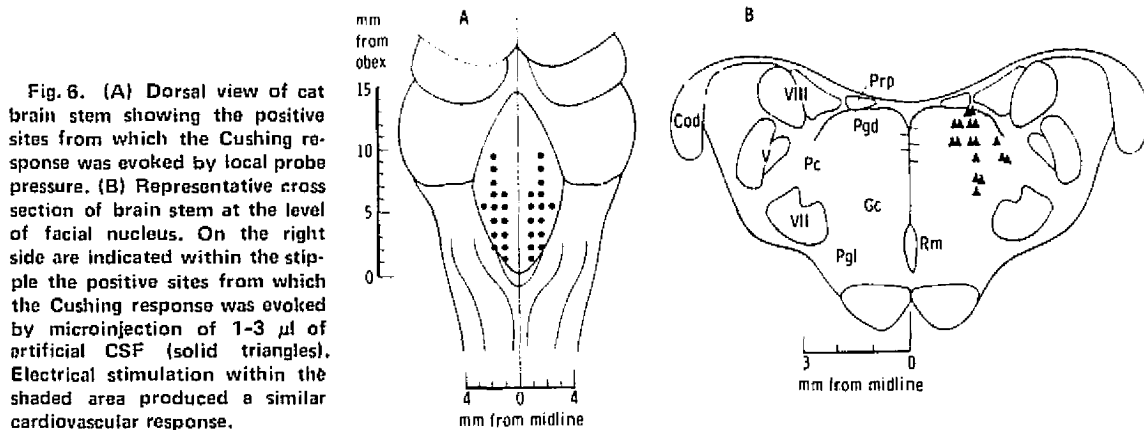
That an acute elevation of intracranial pressure results in a rise of the systemic arterial pressure and a fall in heart rate has been recognized since the middle of the 19th century. In 1902, Cushing⁷⁰ first demonstrated the quantitative nature of the response, showing it was graded and occurred when the pressure within the head exceeded that of the systolic systemic blood pressure. Since his report, the circulatory response to acutely increased intracranial pressure has been known as the Cushing "reflex," or better (since the stimulus or receptors have not been adequately defined), the Cushing response.⁷¹

The Cushing response, when produced acutely

in experimental animals, appears to result in a widespread activation of sympathetic neurons, resulting thereby in vasoconstriction in most vascular beds.^{72,73} The resulting hypertension with low intensity stimuli initially results from an increase of cardiac output, but, with stronger stimuli, the vasoconstriction is enhanced, the total peripheral resistance rises, and the cardiac output may fall, leading in some instances to heart failure and pulmonary edema.⁷² While the Cushing response results in a release of adreno-medullary catecholamines, they do not contribute to the hypertension, since the elevation of blood pressure produced by increased intracranial pressure remains the same after adrenalectomy (Doba and Reis, unpublished observations).

The nature and localization of the receptive areas initiating the Cushing response have only recently been identified. Hoff and Reis⁷¹ demonstrated that the Cushing response initiated by an intracranial balloon persisted after preptontine decerebration and transection of all cranial nerves. However, it disappeared with transection of the spinal cord at the first cervical segment, demonstrating that the receptive area resided within the lower brain stem. By pressing directly over the external and ventricular surfaces of the lower brain stem with a fine metal probe mounted on a strain gauge transducer and lowered with a micro-manipulator, the areas from which an elevation of blood pressure could be elicited were mapped. It was observed that the cardiovascular pattern characteristic of the Cushing response could be evoked from a limited strip lying along the floor of the fourth ventricle.^{71,73} (Fig. 6) The response had a threshold that was close to systolic blood pressure, was graded, and was of short latency.

In an extension of this study, Doba and Reis⁷³ found that a cardiodynamic pattern identical to the Cushing response could be produced by the rapid delivery of 1-3 μ l of artificial CSF by a cannula placed in a restricted area within rostral medulla and caudal pons lying deep to the probe sensitive areas on the surface of the fourth ventricle (Fig. 6). The adequate stimulus appeared to be a transient distortion of tissue within the critical area, since (1) injection of a larger volume in adjacent areas failed to produce a response; (2) the response could not be evoked when fluid was delivered slowly; and (3) the response was independent of the chemical composition of the



injectate. The threshold for the response ranged from 10–30 cm H₂O, which was 5%–10% of the threshold pressure required by application of a probe to the floor of the fourth ventricle. A similar response could also be evoked by punctate electrical stimulation restricted to the same area of the brain stem. Of particular interest in this experiment was the fact that the “pressure” stimulus required for a marked elevation of blood pressure was within the range of capillary pressures and indicates that such a response does not need large distorting pressures on the neuron.

In addition to receptive areas of the lower brain stem, Hoff and Reis⁷¹ discovered that when the intracranial pressure was elevated by injecting artificial CSF under pressure into the lumbar subarachnoid space, the Cushing response was not abolished by cervical cord transection. This suggested that regions of the spinal cord may also participate in the Cushing response. Sensitive sites in the spinal cord were identified by direct probing. These were localized to the cervical and thoracic segments of the cord.

The nature of the stimuli evoking the Cushing response and the identity of the receptive elements in the brain stem remain unsettled. It is unlikely in view of the brief latency, the low threshold, and the dependence on rapid transience of pressure that the response is triggered by local ischemia.^{71,73,74} The bulk of evidence favors the view that the adequate stimulus for the Cushing response is stretch of receptive elements in the brain and/or spinal cord by tissue distortion, either by direct pressure or by axial displacement of the brain stem, as for example, with intracranial masses.^{75,76} The absence of any morphologically distinct “receptors” raises the question as to

whether or not the receptive elements may not be processes of neurons themselves.

Recently, Doba and Reis have discovered that small lesions placed at the caudal extent of the Cushing-sensitive region result in a fall of systemic blood pressure to the same levels to which the blood pressure falls with cervical cord transection (Doba and Reis, unpublished observations). This observation raises the question as to whether or not the Cushing region may be coextensive, if not identical, with the so-called vasomotor area of the lower brain stem whose integrity is required for the maintenance of normal levels of blood pressure.^{11,77}

Acutely increased intracranial hypertension in man leading to distortion of the brain stem are well known to produce hypertension. While the relationship between acutely elevated intracranial pressure and hypertensive disease is remote, it is conceivable that a disorder within these regulating areas may be relevant to chronic hypertensive disease.

Hypertension With Chronically Elevated Intracranial Pressure: The Kaolin Model

In 1932, Dixon and Heller⁷⁸ demonstrated that the injection of kaolin into the cisterna magna produced a moderate but sustained hypertension in experimental animals. The syndrome has been reproduced by others in rats, rabbits, and dogs.^{78–83} The elevation in blood pressure occurs with a latency measured in days, consists of elevation of systolic, diastolic, and mean pressures, and persists after adrenalectomy. It is thus probably neurogenic and not a consequence of release of adrenomedullary catecholamines or adrenocortical steroids.

The mechanisms through which kaolin produces hypertension are not fully understood. It is established that kaolin produces an elevation in the intracranial pressure⁸³ as a consequence of a chronic meningitis produced by the agent. The subsequent meningeal reaction leads to a decreased resorption of CSF and a blockade of flow of spinal fluid out of the fourth ventricle. This latter event may result in a relatively selective increase in distorting pressure on the fourth ventricular floor, producing a chronic Cushing response. Other agents such as thorotrast have also been used to produce hypertension in this manner. It should be emphasized that kaolin and other agents have been used frequently to produce experimental hydrocephalus in animals. In only a few of these studies, however, has the blood pressure been measured, and it is unknown whether such other agents producing chronic hydrocephalus produce changes in cardiovascular function.

In man, chronic elevation of intracranial pressure is probably not itself associated with any elevation in blood pressure (see Dickinson²). Although in the early decades of this century, when advanced neuroradiologic methods were not available, it was hoped that the Cushing response might be a useful diagnostic sign of elevated intracranial pressure, the surmise has not been sustained. The elevation of blood pressure with increased chronic intracranial pressure is probably more related to the location of the lesion and the nature and location of distorting forces than to increased intracranial pressure per se. However, in a widely cited study, Kety et al.⁹³ demonstrated a direct correlation between systemic blood pressure and intracranial pressure in a nonselected group of patients with cerebral tumor. While the data is convincing, the possibility remains that some of the patients with elevated intracranial pressure in whom the arterial pressure was raised had pathologic processes selectively involving posterior brain segments (see below).

Hypertension With Chronic Local Distortion of the Brain Stem

Tumors lying close to the fourth ventricle, particularly tumors of cerebellum can produce significantly elevated arterial pressures in man.⁸⁴⁻⁸⁶ Several cases have been described in which mass lesions, either of the cerebellum or of nearby cranial nerves, distorting the brain stem, were

associated with labile hypertension. In some instances, these simulated the symptoms of pheochromocytoma. In a recent report by Evans et al.,⁸⁶ the diagnosis of pheochromocytoma was entertained in a patient with a posterior fossa mass. Not only were there paroxysmal episodes of hypertension, but the excretion of urinary catecholamines was also markedly elevated. Selected catheterizations did not reveal a localization of the source of catecholamines to the adrenals, and following removal of the cranial mass, hypertension disappeared. It has been suggested that intense fluctuating hypertension in neurologic disease may be a useful diagnostic sign for localization of masses impinging on the floor of the fourth ventricle.⁸⁷

NEUROGENIC HYPERTENSION WITH CEREBRAL ISCHEMIA

Rendering the brain stem acutely ischemic either by ligation of all the major cerebral vessels or by isolated perfusion of the brain can produce a marked reflex elevation of the systemic blood pressure. This cerebral ischemic response^{2,88,89} appears to be unrelated to baroreceptor reflex mechanisms and presumably arises from excitation of receptive areas within the lower brain stem. Since specific receptors have not been identified, it is conceivable that, like the Cushing response, the receptors may be neurons. The acute pressor response to ischemia is associated with an increase in total peripheral resistance and heart rate as well as atrial and ventricular contraction.⁸⁹ The response can be evoked by hypoxia, hypercarbia, or very low perfusion pressure within the cerebral circulation (less than 30-40 mm Hg)^{88,89} alone or in combination. The stimuli are additive,⁸⁹ but it is most likely that when produced by ligation of extracerebral vessels the principal stimulus is chemical. Under conditions of systemic hypoxia the pressor response is additive with those elicited from peripheral chemoreceptors differing only in the fact that the peripheral response activates the cardiac vagus to produce bradycardia, while the centrally evoked response is associated with acceleration of the heart. While cerebral ischemia will activate the adrenal medulla the blood pressure response is minimally changed by adrenalectomy indicating its primary neurogenic nature.

It is unknown whether the cerebral ischemic response is an alarm system activated only when

cerebral blood flow is dangerously threatened or if it represents an important regulatory system that operates "subliminally" at normal conditions of cerebral perfusion but becomes exaggerated with brain-stem ischemia. The cerebral ischemic response differs from the Cushing response in that the latter is, in all probability, a response to distortion of nervous elements rather than hypoxemia or ischemia. Moreover, the Cushing response is associated with bradycardia. It remains to be determined, however, by lesion studies, whether or not different areas of the brain stem are responsible for each of the responses.

Attempts to produce hypertension by chronic ligation of all cerebral arteries have with few exceptions been unsuccessful (for a review of this literature, see Dickinson²). The results in the hands of many investigators have been variable and the hypertension when produced usually transient. Difficulties in assessing the nature of the stimuli have been prominent. The possibilities that surgery can damage the carotid sinus nerves, that ligation of the carotid arteries can produce hypertension through altered peripheral baroreceptor input, and that cerebral ischemia may not be complete, have led to conflicting findings.

An imaginative hypothesis that chronic or recurrent intermittent medullary ischemia can lead to the development of neurogenic hypertension and subsequently to a fixed hypertensive state in man has been proposed by Dickinson.² This interesting conceptualization has, however, at the present not been borne out by experimental evidence.

HYPERTENSION WITH ELECTRICAL STIMULATION OF THE BRAIN

Acute Stimulation

It has been recognized since the end of the 19th century that electrical stimulation of the brain can elicit an elevation of the systemic blood pressure. The development of advanced techniques in instrumentation for stereotaxic placement of electrodes within restricted brain regions, remote stimulation of the brain in association with the measurement of a wide range of cardiovascular events in chronically prepared, freely moving animals, and the recording of bioelectric activity concurrent with the evocation of cardiovascular events have added immeasurably to our knowledge of the central nervous regulation of the circulation.

It is beyond the scope of this paper to review the extensive literature relating to the effects of electrical stimulation of the brain on circulatory function. Certain general principles discussed in detail elsewhere,¹¹ however, may be summarized.

(1) The sites in brain from which blood pressure can be elevated by electrical stimulation are restricted to specific neuronal systems, i.e., a series of neurons that form a close synaptic relationship with each other. While such systems may be highly discrete at any cross-sectional level of the brain, they are represented at all levels from cerebral cortex to spinal cord over the longitudinal axis of the nervous system.

(2) The elevation of blood pressure evoked by electrical stimulation is almost always chronologically related to the stimulus or immediate post-stimulus period. In some instances, poststimulus effects represent the expression of a prolonged epileptic after discharge.⁹⁰

(3) Every circulatory function demonstrated by other methods to be influenced by neural control can be produced by brain stimulation. Indeed, selective changes in isolated vascular beds evoked by localized brain stimulation suggest that part of the organization of the circulation of the brain may be organ specific,^{91,92} and even function specific.

(4) Since selective stimulation of the brain can modify a wide range of cardiovascular parameters, it is no surprise that blood pressure can be elevated with brain stimulation either by increasing the cardiac output or by altering the total peripheral resistance.

(5) Electrical stimulation of the brain at sites from which in chronically prepared unanesthetized animals specific behaviors can be evoked usually will also elicit the appropriate pattern of circulatory changes when the behavior appears naturally.^{10,11} Moreover, stimulation at the same site when the animal is paralyzed will evoke the cardiovascular changes without the motor behavior.⁹³

(6) Elevation of blood pressure of similar magnitude produced by stimulation at different sites in the brain may be associated with very different patterns of blood flow distribution. This fact is best exemplified by the observations by an experiment of Folkow and Rubinstein.⁹³ These investigators placed electrodes in the hypothalamus of cats at sites from which either the defense reaction or feeding behavior could be elicited

when the animal was unanesthetized and chronically prepared. When the animal was anesthetized, stimulation at each site produced an elevation of blood pressure. However, the changes in blood flow associated with stimulation at the site from which the defense response was evoked produced, characteristically, an increase in muscle blood flow and a decrease in splanchnic blood flow; stimulation at the feeding site produced opposite effects on blood flow.

(7) Electrical stimulation can produce elevations of blood pressure through several mechanisms. Electrical stimulation of the brain can directly excite sympathetic preganglionic neurons. Also, it can alter the setting of baroreceptor reflexes in the direction of altering the set point to favor the development of blood pressure elevation. Electrical stimulation of the brain has also been demonstrated to modulate the sensitivity of baroreceptors in the carotid sinus perhaps through the sympathetic innervation of that end organ.

Hypertension With Seizures

During an epileptic seizure of the major motor type (grand mal) occurring either in man or in experimental animals, there is a marked transient elevation in the arterial blood pressure.^{94,95} The elevated blood pressure results, at least in the cat, from a marked increase in total peripheral resistance, with vasoconstriction occurring in renal, femoral, and mesenteric arteries (Doba, Reis, and Beresford, unpublished observations). While organ blood flow is reduced in limbs and viscera, it is actually increased in brain due to the arrest of cerebral autoregulation during the seizure and a passive increase in flow. The hypertension is not a consequence of body movement nor due to release of adrenomedullary catecholamines. Its functional significance probably relates to the need to increase the delivery of oxygen and metabolites to brain in the face of the increased metabolic demands imposed by the seizure. The hypertension associated with a seizure is transient, and there is no evidence that recurrent attacks will lead to chronic hypertension.

Experimental Hypertension With Chronic Brain Stimulation

Since systemic blood pressure can be elevated by acute stimulation, several investigators have sought to determine whether or not chronic re-

peated stimulation of brain can lead to enduring change in the arterial pressure. Attempts to produce hypertension by chronic brain stimulation have rarely been successful, however. In 1947, Taylor et al.⁹⁶ attempted to produce hypertension in dogs by heating the brain stem through a wire embedded in the floor of the fourth ventricle. These attempts to establish "permanent hypertension" by prolonged or repeated stimulation were unsuccessful, failure being attributed to the development of glial scarring around the electrode. In 1951, Taylor and Page⁹⁷ were able to produce chronic hypertension that lasted for as long as 10 mo by combining electrical stimulation of the brain with the production of cerebral ischemia by successively ligating all of the vessels to the brain. It is obvious that this form of hypertension has little relation to that which may occur under normal circumstances in man.

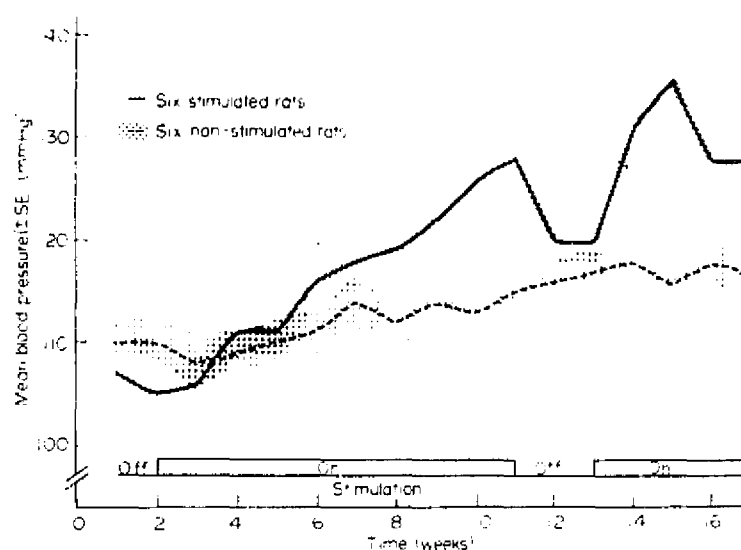
A study with perhaps more interesting physiologic overtones was that of Folkow and Rubinstein.⁹⁸ In this experiment, the investigators implanted electrodes in the lateral hypothalamus of rats at sites from which the cardiovascular components of the defense reaction could be evoked. Six animals were successfully stimulated repeatedly with 10-sec trains once a minute for 12 hr a day over 17 wk. The results depicted in Fig. 7 demonstrate that the stimulated animals gradually developed arterial hypertension that was reversible when stimulation stopped. The stimulus did not produce any obvious changes in behavior. At death, no pathologic changes were observed in the heart or kidneys.

The major problem in this experiment, as acknowledged by the authors, is that it is not possible to ascribe the development of hypertension only to neural activation. Endocrine factors could not be ruled out in the development of hypertension. Electrical stimulation at sites evoking the defense reaction will also release corticosteroids and possibly other hormones. Whether or not such stimulation may lead to chronic functional changes in the blood vessel wall remains to be determined.

NEUROGENIC HYPERTENSION PRODUCED BY BRAIN LESIONS

Hypertension has infrequently been produced by restricted localized lesions of the brain. In the lower brain stem, the neurogenic hypertension due

Fig. 7. Diagram illustrating the gradual change of resting mean blood pressure in rats, which for a prolonged period were exposed to intermittent weak stimulations of the hypothalamic defense area. The control rats were identically treated with exception of the hypothalamic stimulations (By permission.⁹⁸)



to lesions of the nucleus tractus solitarius (NTS hypertension)⁹¹ has already been alluded to and is, to our knowledge, the only experimental study in which brain-stem lesions in otherwise intact animals have produced a sustained elevation of arterial pressure.

Lesions of the hypothalamus have been demonstrated to produce both chronic and acute fulminating forms of hypertension in the rat.⁹⁹⁻¹⁰¹ The chronic form of hypertension has been produced by lesions principally involving the antero-medial hypothalamus. Characteristically, the hypertension develops over several weeks with systolic levels rising to almost 200 mm Hg. It is associated with increased adrenal weight and elevation of cortisol levels and may be abolished by adrenalectomy. This form of hypertension is presumably related to the release of hypothalamic releasing factors resulting in the secretion of ACTH and the development of a steroid-dependent hypertension.

A new syndrome of acute fulminating hypertension in the rat that is not dependent on adrenal cortex has recently been discovered by Nathan and Reis (unpublished observations). The syndrome has been produced by the placement of small bilateral lesions in the anterior hypothalamus of rats anesthetized with halothane. Upon awakening from anesthesia, the animals, after a latency of 30-45 min, begin to develop the gradual onset of arterial hypertension. The hypertension reaches a peak within 90 min and is maintained for several hours until cardiac output falls, the animals de-

velop increasing heart failure, and die of pulmonary edema usually within 6-10 hr. The hypertension in these animals is associated with a marked increase in motor (psychomotor) activity. However, the motor activity is not causal for the arterial hypertension, since the hypertension persists after the animal is paralyzed with curare. At its peak, the hypertension is associated with a marked increase of total peripheral resistance and a decrease of the cardiac output. In this regard, it appears similar to the hypertension produced by lesions of NTS.⁹¹ However, unlike NTS hypertension, in these animals hypertension but not locomotor activity is abolished by adrenalectomy, adrenal demedullation, or selective denervation of the adrenal glands, thereby demonstrating that the hypertension is due to a selective release of adrenomedullary catecholamines. Hypertension of adrenomedullary origin in otherwise normal subjects is not a recognized human disease and would appear to be an example of an entirely new syndrome in animals. The findings also indicated that structures either originating in or passing through the antero-medial hypothalamus exert a tonic inhibitory effect on adrenomedullary secretion of catecholamines.

In man, hypertension, as a consequence of selective lesions of the brain, is infrequent. In some instances, however, hypertension has been reported to accompany poliomyelitis and other forms of encephalitis.¹⁰²⁻¹⁰⁴ In an interesting study, Baker et al.¹⁰² studied the brains of patients with acute bulbar poliomyelitis dying from

circulatory complications often preceded by arterial hypertension. The distribution of brain-stem lesions in this group of subjects differed from those of patients dying with central respiratory failure. The site of major damage lay within paramedian areas of the bulbar reticular formation corresponding to the paramedian areas in the cat, which receive a heavy input from baroreceptors.³²

HYPERTENSION WITH CHRONIC STRESS

The exposure of animals to prolonged stimuli that are physically or emotionally stressful has been observed by several investigators to produce elevated arterial hypertension.¹⁰⁴⁻¹¹⁰ A variety of stresses have been employed, including exposure of the animals to loud noises (audiogenic stimuli),¹⁰⁴⁻¹⁰⁹ to painful electric shocks delivered to the skin,^{107,108} to forced restraint by immobilization,^{108,109} to exposure to cold,¹⁰⁸ or to a series of social challenges—so-called psychosocial stress.¹¹⁰ Characteristically, the hypertension develops over weeks of repeated stress and may persist for days, weeks, or even months after the termination of the stimuli. With daily recurrent stresses, hypertension may persist during the periods between stimulation. The increase in blood pressure is associated with evidence of acute and chronic excitation of the sympathomedullary axis. There is increase in the enzyme dopamine- β -hydroxylase in serum, increased urinary catecholamines, and chronic increase in the activity of the enzymes involved in the biosynthesis of catecholamine in the adrenal medulla, including tyrosine hydroxylase, dopamine- β -hydroxylase, and phenylethanolamine-N-methyltransferase.^{109,111,112}

However, the possibility that chronic adrenocortical activation, changes in dietary intake and distribution of electrolytes, or neurogenically mediated alteration of renal blood flow leading to changes in the production of angiotensin cannot be excluded. Hence, hypertension when produced by stress cannot be considered as purely neurogenic in origin.

HYPERTENSION WITH LEARNED RESPONSES

It has long been known that a transient elevation of systemic blood pressure can be produced during classical (i.e., Pavlovian) conditioning (see Herd et al.).¹¹³ Thus, the presentation of food or

painful electric shocks, which will themselves produce an elevation in the blood pressure (the unconditioned stimulus), can be paired with a light or tone (the conditioned stimulus). After a suitable period, the conditioned stimulus will produce the desired effect. The elevation produced in this manner, however, is rarely of impressive magnitude and is usually transient.

Recently the pioneering studies of Miller and associates¹¹⁴ have shown that the autonomic nervous system, in general, and the blood pressure and heart rate, specifically, can be modified by operant conditioning procedures. Attempts to produce elevated arterial pressures, by operant conditioning have been successfully achieved.^{113,115-117} In an elegant series of studies, Herd and associates^{113,115} trained chronically prepared squirrel monkeys to press a key that turned off a light coupled with the delivery of a noxious stimulus. As the training of the animals progressed and each animal began to press the key more rapidly, the number of noxious stimuli delivered decreased, but the mean arterial pressure rose. Eventually in four of six monkeys, the mean arterial blood pressure became elevated before, during, and after each session, even if a noxious stimulus was not delivered. By similar methods, operant conditioning could be used to return the elevated blood pressure to normal levels.

These provocative findings are of obvious importance in attempting to relate stressful life situations to the genesis and maintenance of essential hypertension. However, at the present time, it is not known whether or not the cardiovascular effects are primarily mediated through neural mechanisms or whether humoral factors may also participate.

SOME BIOCHEMICAL MECHANISMS IN NEUROGENIC HYPERTENSION

Central Catecholamine Neurons and Neurogenic Hypertension

While it has been recognized for many years that norepinephrine is present within the brain, the development of specific histochemical fluorescence methods for the identification of catecholamines in situ have demonstrated the presence in brain of a specific system of neurons that synthesize, store, and release the catecholamine neurotransmitters, dopamine and norepinephrine.^{118,120} The neurons that synthesize dopamine have their cell bodies

located in the upper brain stem, largely in the substantia nigra sending their terminals rostrally to innervate the corpus striatum and possibly the cerebral cortex. In contrast, the system of neurons that synthesize norepinephrine has its cell bodies located in more caudal portions of the brain stem, particularly within specific nuclear areas of the pons and medulla. The noradrenergic neurons send their axons in specific bundles rostrally to innervate the cerebral cortex, basal forebrain areas, the hypothalamus, and varying portions of the upper brain stem as well as sending projections caudally into the spinal cord. Of interest is the fact that the area of NTS is densely innervated by noradrenergic terminals as well as some noradrenergic cell bodies. The cell bodies of noradrenergic neurons function to synthesize the enzymes and subcellular organelles necessary for the synthesis of the amine. These products are then transported distally to the axon terminals, wherein the neurotransmitters are synthesized from tyrosine through a series of specific enzymic reactions, are stored, and are subsequently released by nerve impulse activity.

That central noradrenergic mechanisms may play a role in blood pressure control has been indirectly suggested by the observations that states of behavioral excitement, such as sham rage or the defense reaction in the cat, are characterized not only by elevated blood pressure but also by a markedly increased activity of central noradrenergic neurons.¹²² There is also increasing evidence that some centrally acting agents effective in lowering the systemic blood pressure through interaction with catecholamine neurotransmission, such as clonidine, may do so through central rather than peripheral mechanisms.¹²²⁻¹²⁴

In 1971, Chalmers and Wurtman¹²⁵ discovered that the turnover (i.e., release) of norepinephrine was increased in the spinal cord and hypothalamus of rabbits made hypertensive by sinoaortic denervation. The increase of norepinephrine turnover in the spinal cord was associated with a doubling of the activity of tyrosine hydroxylase, the rate-limiting step in catecholamine biosynthesis. This observation suggested that in some manner baroreceptor input was holding the nerve impulse activity of some system of noradrenergic neurons in check and that consequent to the denervation the activity of these neurons was enhanced. This observation raised the interesting question as to

whether selective interruption of central noradrenergic pathways might attenuate the neurogenic hypertension produced by denervation of baroreceptor afferent fibers either peripherally or within the CNS.

The next year, Chalmers and Reid⁶⁶ showed that the neurogenic hypertension produced by sinoaortic denervation in rabbit could be aborted by the prior injection of the drug 6-hydroxydopamine (6-OHDA) intracisternally in the rabbit. Since 6-OHDA administered in this manner selectively destroyed central but not peripheral catecholamine neurons, the experiment suggested that central noradrenergic projections, possibly those innervating the spinal cord, were required for the expression of arterial hypertension produced by sinoaortic denervation. Recently, Doba and Reis⁶⁹ have shown that in the rat intracisternal 6-OHDA will also abort the development of hypertension produced by NTS lesions. In these experiments, 6-OHDA only produced a significant fall of NE concentrations in the spinal cord. This observation further reinforces the view that neurogenic hypertension produced by central or peripheral deafferentation of baroreceptors is facilitated by bulbo-spinal noradrenergic projections.

On the other hand, it is likely that not all noradrenergic systems facilitate the arterial pressure: The local injection of 6-OHDA into the NTS produces a transient hypertension.⁶⁹ Indeed, this finding suggests that some noradrenergic systems may serve to reduce the blood pressure. The NTS and adjacent medial-dorsal regions of the medulla in the rat are richly innervated with noradrenergic terminals and also contain some cell bodies of noradrenergic neurons. The role of this noradrenergic innervation of NTS is unknown. However, recent studies on the pharmacologic action of the centrally acting hypotensive agent, clonidine, a drug believed to act as an α -adrenergic agonist,¹²³⁻¹²⁴ have suggested that norepinephrine may produce its hypotensive actions by activation of baroreceptor pathways, possibly within the NTS.¹²⁴ Thus, it is conceivable that the single neurotransmitter, norepinephrine, may have opposing central actions on the arterial pressure depending on the site at which it is released, the origin of the parent cell body, and, possibly, the nature of the receptor. Present evidence suggests that in NTS, norepinephrine opposes while in the spinal cord it facilitates a rise of blood pressure.

There is recent evidence suggesting that central noradrenergic mechanisms may participate in the expression of other modes of experimental hypertension. A decrease of norepinephrine turnover has been observed in rats with DOCA/NaCl hypertension¹²⁷ as well as changes of brain-stem catecholamine metabolism in spontaneously hypertensive rats.¹²⁶ Recently, Haeusler and associates¹²⁷ have examined the effects of 6-OHDA administered intraventricularly on several models of hypertension in the rat including DOCA/NaCl hypertension, renal hypertension, and the genetically predetermined spontaneous form of hypertension (SHR). The administration of 6-OHDA did not permanently reverse the elevation of blood pressure in any of these animals. On the other hand, treatment prior to the induction of DOCA/NaCl or renal hypertension or before the age at which spontaneously hypertensive rats demonstrate the elevated blood pressure prevented the expected rise. These investigators have suggested that central trigger mechanisms dependent on a catecholaminergic innervation in the brain may initiate a sequence of events possibly leading to changes in the arterial wall and hence to hypertension.

Angiotensin and Central Cardiovascular Control

In the past few years, evidence has accrued to indicate that the octapeptide angiotensin II (angiotensin) may exert some, if not the greater part, of its cardiovascular effects indirectly through central neural actions. The central actions of angiotensin have recently been comprehensively reviewed¹²⁸ and may be summarized. The evidence for a central role of angiotensin includes the findings that when infused into the vertebral artery, angiotensin has a greater hypertensive action than when administered intravenously,¹²⁹⁻¹³¹ and that a hypertensive effect can be evoked by intraventricular or focal intracerebral administration. Several sites for the central action of the agent have been proposed on the basis of injections and/or lesions. These include a region of the lower brain stem adjacent to NTS, the area postrema, which is devoid of a blood brain barrier, and a site within the midbrain. In addition, the demonstration that renin and angiotensin are endogenous to brain, and detection of an angiotensin-I-like protein in the cerebral spinal fluid of hypertensive man have added evidence to suggest that angio-

tensin may play some role in human hypertensive disease.

The mechanism of action of angiotensin in the brain is unknown. However, it is possible that angiotensin may act indirectly through central noradrenergic systems. The evidence for this is in part a demonstration that the central dysogenic action of angiotensin when introduced directly into the brain by a microcannula, depends upon the release of catecholamines.

Of particular interest in regard to the interaction of the CNS with angiotensin is the fact that sympathetic nerve discharge producing renal vasoconstriction can result in an increased level of angiotensin in the blood. It would thus appear that a centrally mediated increase in neurally mediated renal vasoconstriction could produce a release of renin, augment angiotensin levels, and thus produce a positive feedback on vasomotor activity in the brain. This mode of regulation would be opposite to the negative feedback exerted by baroreceptors. The manner in which these two inputs interact in the regulation of central vasomotor control is not known.

Cyclic Nucleotide Metabolism in Blood Vessels and Heart in Neurogenic Hypertension

It has been proposed that an increase in the discharges of sympathetic neurons when sustained can lead, over time, to structural and/or biochemical changes in blood vessels.^{2,9} These changes may "fix" the hypertension such that a subsequent resumption of normal levels of sympathetic discharge will not reverse the increase in vascular resistance and the blood pressure. Such "transmutation" of a transient to a fixed state may be a link between neurogenic and essential forms of hypertension.

The mechanisms by which the nerve impulse activity is transduced to effect changes in the arterial wall or vascular receptors are not understood. One possible mode of action would be through an effect on the metabolism of cyclic nucleotides in blood vessel walls. The cyclic nucleotide system might, therefore, be an important interface between the sympathetic nerves and blood vessels, since the levels of the cyclic nucleotides, cyclic adenosine monophosphate (AMP), and cyclic guanosine monophosphate (GMP) appear to play a critical role in determining the tone of smooth muscle contractility (see

Amer¹³²). Moreover, the metabolism and accumulation of these nucleotides may be altered by catecholamine neurotransmitters.

Amer¹³² has recently demonstrated that the aortas from spontaneously hypertensive rats, or rats in whom hypertension was produced by prolonged stress, contain significantly lower concentrations of cyclic AMP than did their respective controls. The reduced AMP levels resulted from increased activity of the degrading enzyme, phosphodiesterase. Amer postulated that a reduction of cyclic AMP in blood vessels could be a common mechanism for the increased arteriolar resistance in both forms of hypertension.

Recently, Reis, Doba, and Amer¹³³ have studied changes in the metabolism of cyclic nucleotides in aortas and hearts of rats in whom acute neurogenic hypertension was produced by bilateral lesions of NTS. The tissues were obtained from the animals 2 hr after the placement of lesions at a time when the hypertension was elevated, marked, and maintained. During acute neurogenic hypertension, it was observed that there was a significant increase in the levels of cyclic AMP and a reduction of cyclic GMP in the aortas and hearts of the lesioned but not sham-operated animals. The decreased cyclic AMP was due to increased activity of the high affinity form of phosphodiesterase coupled to a decreased responsiveness of adenylyl cyclase to activation by isoproterenol. In contrast, the increase in GMP was a consequence of activation of the synthesizing enzyme, guanylylcyclase. The changes in cyclic nucleotide metabolism appear to be due to catecholamine release and not to the hypertension per se (Amer, Doba, and Reis, unpublished observations). (1) Rats, previously treated with systemic 6-OHDA and in whom the adrenal glands were removed just prior to placement of NTS, did not develop the hypertension, nor did they demonstrate changes in the levels of the cyclic nucleotides in their aortas or hearts. (2) Elevation of vascular pressures within the heart by acute ligation of the aorta did not produce the fall of cardiac cyclic AMP that was associated with neurogenic hypertension in the heart. The results therefore, suggest that a very brief activation of

sympathetic neurons can produce biochemical changes in the blood vessels and hearts of the rat that are of the same direction and magnitude as those seen in animals with either prolonged stress or genetically determined forms of hypertension. Conceivably, these changes of cyclic nucleotide metabolism may become fixed, leading ultimately to changes of vascular contractility and, hence, a more permanent change in vascular resistance and elevation of the blood pressure.

SUMMARY AND CONCLUSIONS

It is evident from studies on animals that manipulation of the central neural mechanisms governing the discharge of sympathetic neurons can lead to prolonged and even lethal arterial hypertension. Such neurogenic hypertension can be produced by (1) interruption at peripheral or central sites of reflexes tonically inhibitory to the sympathetic nervous system; (2) elevation of the intracranial pressure, which leads to distortion by stretch/pressure of specific neurons of the brain stem and spinal cord; (3) direct distortion of sensitive regions of the lower brain stem; (4) cerebral ischemia and/or hypoxia; (5) electrical stimulation of specific neural systems that excite sympathetic neurons; (6) selective brain lesions; (7) chronic stress; or (8) learned responses. Moreover, there is increasing evidence that biochemically specific pathways in the brain, notably those that synthesize, store, and release the neurotransmitter norepinephrine may be critical for the expression of increased sympathetic tone. It may be through these sites that agents synthesized peripherally, such as angiotensin, act in regulating vasomotor tone systemically. Indeed, it may also be at these control sites that several of the pharmacologically active agents that are clinically efficacious in controlling hypertension in man may act. Finally, there is increasing evidence that the metabolism of cyclic nucleotides, which are in part related to receptive elements within blood vessels, may be altered by sympathetic discharge, particularly when prolonged, leading to enduring changes in vascular resistance that ultimately may lead to a fixed hypertensive state.

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HYPOXEMIA, ATELECTASIS, AND THE ELEVATION OF ARTERIAL PRESSURE
AND HEART RATE IN PARALYZED ARTIFICIALLY VENTILATED RAT

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(Received in final form March 3, 1975)

Rats prepared while anesthetized with halothane, ether or pentobarbital, subsequently paralyzed with curare, and maintained with or without anesthetic, by artificial ventilation with room air are hypoxemic in association with elevated arterial pressures and heart rates. The hypoxemia can occur with normal PaCO_2 , is associated with a marked increase in the alveolar-arterial PO_2 difference, and is not reversed by hyperventilation or hyperinflation. The lungs, visualized directly through a thoracotomy during artificial ventilation, are segmentally collapsed and at postmortem demonstrate focal and diffuse signs of atelectasis. Hypoxemia and an elevation of the alveolar-arterial PO_2 difference occur within 20 minutes after the onset of anesthesia, prior to paralysis. We conclude that anesthetized rats develop atelectasis soon after the onset of anesthesia. The atelectasis, and resultant hypoxemia persist during subsequent paralysis despite an adequate minute volume and absence of anesthesia. Despite atelectasis, blood gases, arterial pressures and heart rates may be maintained near normal values by ventilation of paralyzed rats with 50% O_2 and 50% N_2 .

In recent years analysis of neural mechanisms underlying conditioned autonomic responses in rat, has led to increasing use of this animal in the paralyzed artificially ventilated and unanesthetized state (16,30). While it is usually assumed that with adequate ventilation the paralyzed animal has normal blood

gases, during recent studies of the neural control of cardiovascular system we found that artificially ventilated rats were generally hypoxemic and that the hypoxemia was associated with abnormally high arterial pressures and heart rates. Since hypoxemia is known to produce similar effects in the dog (11,19,25), we sought to determine if such is also the case in the rat, and if so, to seek a simple and practical means of ensuring normoxia, and therefore, normal arterial pressure and heart rate. We report that hypoxemia is responsible for the elevation of arterial pressure and heart rate, that it is due to atelectasis, and that it is correctable by ventilating the animal with a suitable gas mixture. Such information should be of practical importance to others whose studies require the use of the paralyzed, artificially ventilated, and unanesthetized rat.

Methods

Animals. The experiments were performed on Sprague-Dawley rats of both sexes (Carsworth Farms) weighing 290-450 gm. The animals were housed in groups of 6 in light-cycled, thermally-regulated rooms and permitted free access to food and water.

Procedures. One group of animals served as their own controls for assessment of the effects of artificial ventilation on blood gases by comparing the blood gases measured in the animals when awake and unrestrained and when subsequently paralyzed and artificially ventilated. The animals were first anesthetized with halothane (2-3% in 100% O₂) blown over the nose through a face mask. A polyethylene cannula (PE 50) was placed in the right common carotid artery, fixed to soft tissue and the other end brought out through the skin at a point midway between the scapu-

lae. A saddle (LeHigh Valley Electronics #191-10) secured to the animal by a strap protected the cannula. The anesthetic was discontinued and the animal was placed in a standard rat cage (47 x 20 x 25 cm). The cannula was threaded through a flexible metal spring which was attached at one end to the saddle and at the other end to a hydraulic swivel (LeHigh Valley Electronics #192-03) mounted on the top of the cage. The swivel permitted the animal to rotate in a full circle without twisting the cannula. A stopcock connected to the cannula permitted sampling of arterial blood or connection to a transducer (Stathem, P23Db) for display of arterial pressure on a polygraph (Beckman, type RM). Heart rate was computed from the arterial pressure pulse wave by a cardiometer (Beckman 9857) and was simultaneously displayed.

By 15 minutes after cessation of anesthesia the animals were awake and exhibiting levels of movement comparable to unoperated controls. Movement of the animals across the cage was detected by interruption of a beam of light focussed on a photo-cell detector. The output of the photo-cell was connected to an electro-mechanical counter. Each interruption of the light beam produced a pulse at the output of the photo-cell device that increased the current count by one. Blood samples were withdrawn at 60 minutes after termination of the anesthesia. The animals appeared unaware of the sampling procedures.

Following the sampling, the animals were anesthetized again with halothane and in preparation for paralyzation and artificial ventilation, a tracheal tube was inserted and the right femoral vein was cannulated. Movement was monitored by electromyographic recordings of activity of leg muscles and displayed on a polygraph.

Other animals, from which no prior blood gas or cardiovascular measurements had been made were anesthetized with either halothane, diethyl-ether in air (administered through a nose cone containing a sponge saturated in ether), or sodium pentobarbital (40 mg/kg, i.p.). In these animals the trachea, right femoral vein and right common carotid arteries were also cannulated.

At the end of the surgical procedures the halothane or ether anesthesia was discontinued and all the animals were paralyzed with curare (0.4 - 0.8 mg/kg, i.v.) and artificially ventilated by a small animal respirator (Harvard Apparatus Co., #680). The pressure generated by the pump was measured by a T tube at the mouth of the animal varied between +8.79 cm of water in inspiration and -0.21 cm of water in expiration. The pump was adjusted to provide an average tidal volume of 1.75 cc at a respiratory frequency of 80/min (average minute volume of 140 cc). The tidal volumes were selected according to body weight by use of the nomogram of Kleinman and Radford as supplied by the Harvard Apparatus Co.

All animals were ventilated with either room air or 50% O₂ and 50% N₂ for 45 minutes. The animals ventilated with room air were then subjected to one of the following conditions: 100% O₂ for 15 min, hyperventilation for 15 min, or periodic hyperinflation of the lungs for 45 min. Hyperventilation was accomplished by increasing the tidal volume to 2.5 cc and the respiratory rate to 100 breaths/min (minute volume of 400 cc). The lungs were hyperinflated with a total volume of 5.25 cc every quarter hour. At 15 minutes prior to sampling, the lungs were hyperinflated every 5 minutes. The volume of 5.25 cc, although representing only about 50% of the total lung volume (1, p.111) was selected since larger volumes frequently resulted in rupture of pulmonary

capillaries.

Great care was taken to insure the comfort of the paralyzed, unanesthetized animals. All wounds were packed with cotton saturated with aqueous procaine (2%). The eyes were covered with procaine ointment (5%). The inspired air was humidified. Body temperature was maintained at 38° ($\pm 0.5^{\circ}$) through the use of a rectal probe connected to a thermostatically regulated electric heating pad.

The animals were killed with an overdose of barbiturate at the end of the experiments. The trachea and pulmonary vessels were quickly clamped while the lungs were inflated by the respirator. The lungs were examined for gross signs of atelectasis and then removed. After a storage period of at least one week in 10% formalin, the lungs, which were still inflated, were cut into longitudinal sections and stained with hematoxylin and eosin for microscopic examination.

Blood Gas Analysis

One ml syringes were prepared for sampling by aspirating heparin solution to lubricate the barrel and expelling all the solution except that which remained in the dead space of the syringe. The cannula was flushed with arterial blood by withdrawing 0.04 ml into a syringe and then 0.2 ml was withdrawn into the sampling syringe for analysis.

No more than three samples (total of 0.6 ml) were taken from any one animal. That amount of blood was estimated to be about 3.0% of the total whole blood volume which is between 20 and 32 ml in 290-450 gm rats (1, p.381). Blood samples that were not analyzed immediately were held in heparinized, sealed glass syringes and stored in ice for no longer than 90 minutes. In control experiments it was found that this procedure did not result

in a PO_2 value which was statistically different from that measured immediately after sampling.

The blood samples were analyzed at $37^\circ C$ for arterial CO_2 ($PaCO_2$), arterial O_2 pressure (PaO_2) and pH by use of a Radiometer Model BMS3. Corrections were made for daily variations in barometric pressure (20). The nomogram of Kelman and Nunn (18) was used to correct for the difference between rectal temperature and the temperature of the water bath containing the electrodes. This correction varies with PO_2 and it was usually 1 or 2%. No correction for length of time between blood sampling and analysis was necessary since the samples were immediately placed in ice following withdrawal (18). The O_2 and CO_2 electrodes were calibrated with known gas pressures certified to be accurate within $\pm 0.01\%$. Two calibration points for each gas were used (0% and 21.25% for O_2 ; 4% and 11.94% for CO_2). Our oxygen electrode was found to give readings which were higher in gas than in blood with the same PO_2 by a factor of 1.0095. This factor was used to calculate the PO_2 in blood samples from the reading of the electrode.

Alveolar O_2 partial pressure (PAO_2) was calculated from blood gases obtained while the animals were breathing 100% O_2 according to the following formula (15,17,28,32):

$$PAO_2 = P_B - 49.7 - PaCO_2$$

where P_B is barometric pressure and water vapor pressure = 49.7 mmHg at $38^\circ C$. The alveolar-arterial O_2 pressure difference was calculated as $PAO_2 - PaO_2$.

Statistical Evaluation

Because of the relatively small number of animals in some

groups, non-parametric tests of significance were used. Two-tailed tests of significance ($p < .05$) utilized the Walsh test for paired samples and the Mann-Whitney U-test for unpaired samples (29). For paired comparisons of more than 15 observations the t-test was used (31).

Results

Blood Gases in Paralyzed and Artificially Ventilated Rats Displaying Elevated Arterial Pressures and Heart Rates

Paralyzed, unanesthetized and artificially ventilated rats displayed elevated arterial pressures and heart rates (Fig. 1) in association with hypoxemia (Table 1). The PaCO_2 was only minimally elevated and it was therefore unlikely that the hypoxemia was due to hypoventilation. Further support for this argument was supplied by the fact that when the minute volume was increased 2.6 times to 400 cc, the PaO_2 was raised to only 71 mmHg, and at the same time hypocapnia and a marked respiratory alkalosis occurred (Table 1).

The persistence of hypoxemia, despite hyperventilation, suggested the presence of a pulmonary arteriovenous shunt (4,15,17). Support for this argument was provided by ventilating the paralyzed animals with 100% O_2 and noting the large difference in alveolar and arterial O_2 pressures ($\text{PAO}_2 - \text{PaO}_2$) in comparison to the small difference observed in unrestrained controls that spontaneously breathed 100% O_2 (Table 1).

Time of Onset of Atelectasis

To determine if the onset of atelectasis and hypoxemia occurred prior to paralysis, we examined the blood gases 20 minutes after the initiation of 2% halothane anesthesia (in 100% O_2)

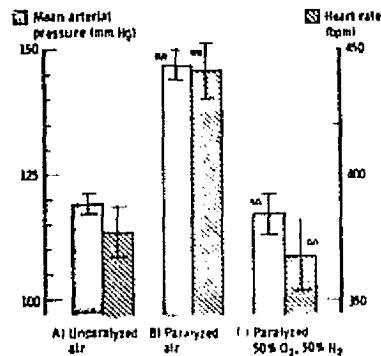


FIG. 1

Effect of Hypoxemia on Arterial Pressure and Heart Rate. Arterial pressure and heart rate were measured in rats ($n = 19$) that were unparalyzed and spontaneously ventilating with room air (A). When the animals were subsequently paralyzed and artificially ventilated with room air they became hypoxemic and showed elevated arterial pressures and heart rates (B). Other rats ($n = 8$) were paralyzed, but artificially ventilated with 50% O₂ - 50% N₂ (C). These animals were normoxic and had arterial pressures and heart rates comparable to those of the animals that were unparalyzed and spontaneously breathing room air.

** $p < .001$ differs from the unparalyzed-air group.

$\Delta\Delta p < .001$ differs from the paralyzed-air group.

when the animals were breathing spontaneously. At this time the animals were obviously hypoventilating since they were profoundly hypercapnic (Table 2) and the alveolar-arterial P_{O₂} difference was unusually high. The lungs of animals killed at this time were atelectatic indicating that atelectasis preceded paralyzation.

Pathological Changes in the Lungs of Artificially Ventilated Rats

Atelectasis appeared to be the most likely cause of acute pulmonary shunting for two reasons: first, after thoracotomy, direct visual inspection of the lungs in anesthetized, paralyzed and artificially ventilated rats revealed an obvious partial collapse of the lungs, particularly of the upper lobes. The collapsed lobes could not be expanded by hyperinflation unless adjoining aerated areas were inflated to such an extent that the lung volume exceeded that of the open thoracic cavity.

TABLE 1
Blood Gases and Alveolar Arterial Oxygen
Differences in Unanesthetized Paralyzed Rats

Ventilation Condition	Inspired Gas	Unrestrained	Paralyzed	
		Spontaneous†	Artificial	Hyperventilated
V_M (cc)†		-	140	400
PaO_2 (mm Hg)	air	92 ± 2 (19)	61 ± 4 (19)***	71 ± 6 (11)**
$PaCO_2$ (mm Hg)	air	34 ± 1 (19)	37 ± 2 (19)	14 ± 1 (11)***
$PAO_2 - PaO_2$ (mm Hg)	100% O ₂	89 ± 13 (7)§	384 ± 15 (6)***	.
pH (units)	air	7.34 ± 0.01 (17)	7.40 ± 0.02 (17)	7.57 ± 0.01 (11)***

* Differs significantly from unrestrained, * $p \leq .05$; ** $p \leq .01$; *** $p \leq .001$. Paired comparisons were made.

† A values expressed as the mean \pm standard error. Numbers in parentheses = number of animals.

† V_M = average minute volume.

§ Values for anesthetized animals from Turek *et al* (32). All other values are from unanesthetized animals of the present study.

Blood samples were taken from unrestrained, freely-moving animals 60 min after termination of halothane anesthesia. The animals were re-anesthetized, a tracheal cannula inserted, paralyzed with curare, artificially ventilated at the normal V_M with room air and anesthetic discontinued. After 45 min a blood sample was taken for determination of PaO_2 , $PaCO_2$ and pH. Six of these animals were then ventilated with 100% O₂ for 15 min for determination of $PAO_2 - PaO_2$. Eleven animals were hyperventilated for 15 min and a blood sample taken.

Second, microscopic examination of the lungs showed the classical signs of atelectasis including the presence of many partially to completely collapsed alveoli in association with capillary congestion (Fig. 2A). There was no evidence of pulmonary infiltration or infection, nor was any abnormal thickening or separation of the capillary and alveolar walls noted.

Effect of Different Anesthetic Agents

We next sought to determine if anesthetics other than halothane could produce atelectasis and hypoxemia by measuring the blood gases in animals anesthetized with ether or sodium pento-

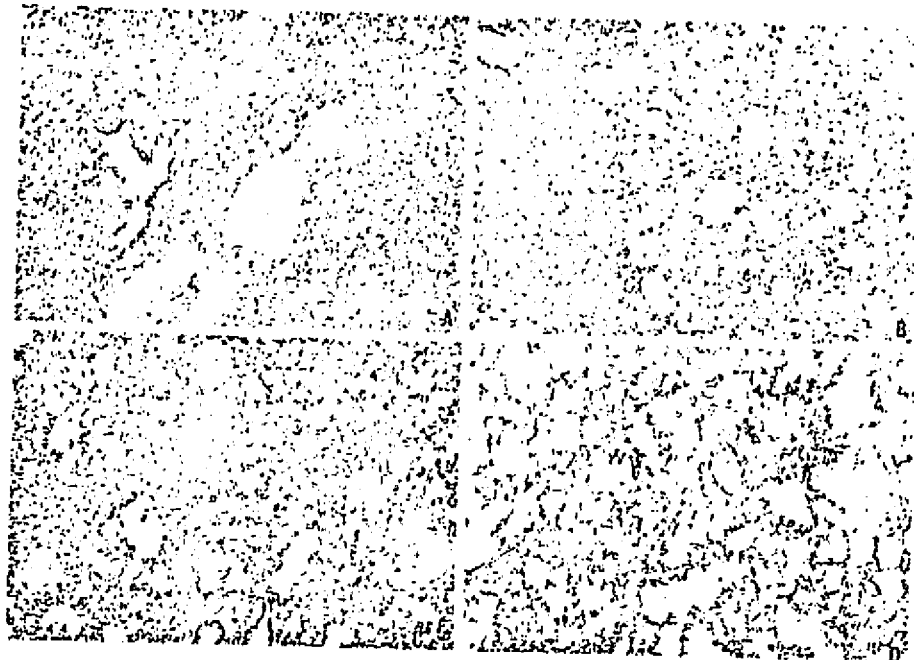


FIG. 2

Longitudinal section of superior lobe of lungs of rats artificially ventilated for 45 minutes on room air following anesthetization with 2% halothane in O₂ (A), ether in air (B) or pentobarbital (C), compared with normal control (D), killed immediately following anesthetization. Note collapsed alveoli and capillary engorgement which is greatest with halothane. (Hematoxylin and eosin, X6.3 magnification).

barbital at: (a) 20 minutes after onset of anesthesia when spontaneously breathing room air, and (b) 45 minutes after paralyzation and artificial ventilation with room air.

The results of the experiments are tabulated in Table 2 and demonstrate several features. First, as when halothane is used, hypoxemia occurred within 20 minutes after administration of ether or pentobarbital at a time when animals were spontaneously breathing room air. The increase in PaCO₂ at 20 minutes after administration of pentobarbital indicated that these animals,

like those anesthetized with halothane, hypoventilated. However, the fact that the animals anesthetized with ether were normocapnic, yet still hypoxemic, indicated that the hypoventilation is not a necessary condition for the occurrence of hypoxemia during anesthesia.

Second, the hypoxemia persisted during artificial ventilation irrespective of the particular anesthetic used during the preparation of the animal. The hypoxemia was also unrelated to the presence or absence of the anesthetic agent during the period of assisted ventilation since it occurred with either the barbiturate or the volatile agents.

Finally, the chemical data indicate that while the identity of the anesthetic agent was not the determinant in producing hypoxemia, halothane appeared to produce a more severe syndrome than did ether or pentobarbital. Thus, animals anesthetized with halothane were more hypoxemic, exhibited a greater elevation in the alveolar-arterial PO_2 difference (Table 2), and a more intense degree of atelectasis (Fig. 2) after 45 minutes of artificial ventilation than animals anesthetized with ether or pentobarbital.

Correction of Hypoxemia and Establishment of Normal Arterial Pressures and Heart Rates in Paralyzed Artificially Ventilated Rats

We attempted to correct the abnormalities in blood gases in the paralyzed, artificially ventilated rats by two methods. First, the use of the common procedure of intermittently increasing the end-expiratory pressure by hyperinflation (3,5,12,22) raises the PaO_2 only slightly to levels which were still subnormal (Table 3) and the procedure also produced hypocapnia.

The second technique consisted of artificially ventilating the rat with a gas mixture of 50% O_2 and 50% N_2 at an average

TABLE 2
Effect of Different Anesthetics on Blood Gases Before and After Paralyzation

Restraint Condition	Anesthetic						
	No Anesthetic	Halothane (in 100% O ₂)		Ether (in air)		Pentobarbital†	
	Unrestrained	Anesthetized	Paralyzed	Anesthetized	Paralyzed	Anesthetized	Paralyzed
Ventilation Condition	Spontaneous	Spontaneous	Artificial	Spontaneous	Artificial	Spontaneous	Artificial
V _T (cc)	----	----	140	----	140	----	140
PaO ₂ (mm Hg)	92 ± 2 (19)	167 ± 13 (5)	61 ± 4 (19)***	72 ± 6 (7)*	74 ± 3 (4)**	64 ± 2 (5)**	73 ± 2 (5)*
PaCO ₂ (mm Hg)	34 ± 1 (19)	56 ± 5 (5)**	37 ± 2 (19)	39 ± 4 (7)	31 ± 5 (4)	44 ± 4 (5)*	29 ± 2 (5)*
PAO ₂ -PaO ₂ (mm Hg)	89 ± 13 (7)§	491 ± 17 (5)*	384 ± 15 (6)***	----	326 ± 9 (4)**	----	326 ± 11 (5)*
pH (units)	7.34 ± 0.01 (17)	7.27 ± 0.01*	7.40 ± 0.02 (17)	7.32 ± 0.04 (7)	7.45 ± 0.03 (4)*	7.35 ± 0.02 (5)	7.53 ± 0.03 (5)*

* Differs significantly from no anesthetic, *p<.05; **p<.01; ***p<.001. Paired comparisons were made of values measured from the same animals while unrestrained and later paralyzed. All other comparisons were unpaired.

† All values expressed as the mean ± standard error. Numbers in parentheses = number of animals.

‡ Only the pentobarbital animals were anesthetized during paralyzation and artificial ventilation.

§ From the data of Turek *et al* (32).

Blood samples were taken from 19 unanesthetized, chronically prepared animals after 60 min of spontaneous breathing. These animals were then paralyzed and artificially ventilated for 45 min and another sample was taken. Six of the animals were artificially ventilated for another 15 min with 100% O₂ and a blood sample taken for determination of PAO₂ - PaO₂. Other animals were anesthetized with halothane (in 100% O₂), ether (in air), or pentobarbital (40 mg/kg, i.p.) and allowed to spontaneously ventilate for 20 min. At the end of that time a blood sample was taken for determination of PaO₂, PaCO₂, pH and PAO₂ - PaO₂. These animals were then paralyzed and artificially ventilated for 45 min and another sample was taken. The PAO₂ - PaO₂ determinations were made by ventilating the same animals for an additional 15 min with 100% O₂.

minute volume of 140 cc. This method was successful in elevating the PaO_2 levels while maintaining the PaCO_2 within the normocapnic range (Table 3). The blood gases and the associated arterial pressures and heart rates obtained with this method were thus quite similar to those observed in the spontaneously ventilating, unrestrained controls (Fig. 1, Table 1).

TABLE 3
Effects of Hyperinflation or Administration of
50% O_2 - 50% N_2 on Blood Gases in
Paralyzed Artificially Ventilated Rats

Ventilation Condition	Inspired Gas		
	Air	Air	50% O_2 - 50% N_2
	Normal	Hyperinflation	Normal
\dot{V}_M (cc)*	140	420	140
PaO_2 (mm Hg)	61 ± 4 (19)†	79 ± 2 (4)*	101 ± 11 (14)**
PaCO_2 (mm Hg)	37 ± 2 (19)	28 ± 1 (4)*	38 ± 2 (11)
$\text{PAO}_2 - \text{PaO}_2$ (mm Hg)	387 ± 15 (6)	327 ± 24 (4)*	-
pH (units)	7.40 ± 0.02 (17)	7.46 ± 0.02 (4)	7.37 ± 0.02 (11)

* Differs significantly from air-normal ventilation, * $p < .05$; ** $p < .01$. Paired comparisons were made of values measured in the same animals while normally ventilated with room air or 50% O_2 - 50% N_2 . Unpaired comparisons were made of values obtained in the animals normally ventilated with air to other animals that were hyperinflated with air.

† All values expressed as the mean \pm standard error. Numbers in parentheses = number of animals.

‡ Respiratory frequency (80/min), the same in all groups.

Nineteen chronically prepared, unanesthetized animals were paralyzed and artificially ventilated on room air. Blood samples were taken after 45 min of ventilation at the normal \dot{V}_M . Fourteen other animals were ventilated for 45 min on 50% O_2 - 50% N_2 and blood samples obtained. Four other animals were hyperinflated for 45 min and blood samples obtained. While maintaining hyperinflation the animals were ventilated with 100% O_2 for 15 min and a blood sample taken for determination of $\text{PAO}_2 - \text{PaO}_2$.

Discussion

This study demonstrates that rats, paralyzed and artificially ventilated, are hypoxemic and that the hypoxemia, as in dog, is associated with elevated arterial pressure and heart rate. The

mechanism by which hypoxemia in the artificially ventilated rat is related to the development of a pressor response and tachycardia has not, to our knowledge, been investigated. However, it is well established that in the artificially ventilated dog pressor responses invariably accompany hypoxemia and are usually ascribed to stimulation of the peripheral arterial chemoreceptors (6,9,23,25). The effect of hypoxemia on the direction of the cardiac rate change in the artificially ventilated dog is not so clear-cut. Some have reported that bradycardia is associated with hypoxemia during artificial ventilation (9,23), while others report that tachycardia results (6,11,19). Only tachycardia was noted in the hypoxemic, artificially ventilated rats of the present study and the magnitude of the cardiac acceleration was greater than that observed in dogs at blood oxygen tensions which were the same or slightly lower than those observed by us. The generally larger tachycardias and also pressor responses observed in the rat may reflect the fact that they were unanesthetized whereas all of the dogs utilized in the studies cited earlier were anesthetized.

That the hypoxemia is not a consequence of inadequate tidal or minute volume during artificial ventilation is supported by two observations. First, the hypoxemia is not necessarily associated with hypercapnia or acidosis. Second, hyperventilation fails to elevate the PaO_2 of the paralyzed rats to values that fall within the normal range observed in the spontaneously breathing, unrestrained animals. These findings, in association with the presence of an elevated alveolar-arterial PO_2 difference, provide chemical evidence that the hypoxemia is most likely due to the development of a pulmonary-arteriovenous shunt (4,21). Compromise of O_2 diffusion capacity because of an increase in

diffusion distance is unlikely, since histological examination of the lungs failed to reveal any abnormal thickening or separation of the capillary and alveolar walls. On these chemical and morphological grounds, the shunting appears to be a consequence of a moderate to severe degree of alveolar collapse.

The hypoxemia, the increase in the alveolar arterial PO_2 difference and the atelectasis developed after only 20 minutes of anesthesia, and prior to paralysis and artificial ventilation. That blood gases were normal in the unrestrained and unanesthetized animals 60 minutes after termination of anesthesia indicates that the atelectasis is largely reversible. However, with paralysis the atelectasis persists and, once established, appears resistant to reversal by hyperinflation. The cause of the atelectasis is unknown. While several factors known to facilitate the development of atelectasis in other species including hypoventilation (2,3,33), and suppression by anesthesia of reflex sighing and gasping [which normally can help to open up collapsed alveoli (7,10,14,15,27)] may be contributory, they cannot be the entire explanation. Thus, while rats anesthetized with halothane or pentobarbital hypoventilated, animals anesthetized with ether did not, yet all were equally hypoxemic. All animals were held in a supine or lateral position required for access to the cannulation sites and then they were turned to a prone position during the period of artificial ventilation. Thus, position may have contributed to the production of atelectasis as in larger animals and man (2,3,24,26,28) although Farhi (13) has shown that, because of the small lung dimension of the rat, severity of atelectasis is unrelated to body position.

While all of the anesthetics produced atelectasis and hypoxemia, halothane in 100% O_2 produced the most severe pathologi-

cal and clinical syndrome. Conceivably, two features of this anesthetic contributed to its potent actions. First, halothane, as administered in this study, produced a greater hypoventilation than did ether or pentobarbital which may have increased the severity of the atelectasis. Second, the use of 100% O₂ as the carrier for the halothane probably contributed to the development of the atelectasis because of the large oxygen pressure gradient established between alveolar space and capillary vessels which favors a rapid and complete alveolar collapse (8,12).

The fact that the hypoxemia in paralyzed rats after the onset of paralysis and artificial ventilation was associated with a nearly normal PaCO₂ is a point of practical importance. It highlights the fact that the common practice of judging ventilatory adequacy in paralyzed, artificially ventilated animals by measuring end-tidal PCO₂ may fail to reflect accurately the degree of hypoxemia.

The hypoxemia, although not the atelectasis, may be corrected in paralyzed rats by the simple expedient of ventilating the animals with a mixture of 50% O₂ and 50% N₂ at an adequate tidal volume. By the use of an appropriate gas mixture, it is therefore possible to maintain paralyzed rats over prolonged periods of time with blood gases and consequently arterial pressures and heart rates close to normal levels.

Acknowledgment

The authors thank Dr. Barry Dworkin and Miss Sarah Kirkland for assistance in blood gas determinations early in the study. Dr. William Briscoe provided invaluable criticism of the study. Research was supported by grants from the NIH (NS 08476) and NASA (NGR 33-010-179).

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CHANGES IN REGIONAL BLOOD FLOW AND CARDIODYNAMICS ASSOCIATED WITH ELECTRICALLY AND CHEMICALLY INDUCED EPILEPSY IN CAT

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(Accepted December 14th, 1974)

SUMMARY

Changes in cardiodynamics and regional blood flow were examined in chronically prepared paralyzed cats during seizures induced electrically by transcerebral or direct cortical stimulation or by administration of flurothyl ether (Indoklon) or pentylenetetrazol (Metrazol). Transcerebral and chemical stimuli produced the greatest vascular responses. During seizures there was an abrupt elevation of arterial pressure unassociated with consistent changes in heart rate. Vascular resistance was increased in femoral, renal and mesenteric arteries with variable reductions in blood flow. Resistance was decreased and flow passively increased in the common carotid artery reflecting the loss of cerebral autoregulation. Cardiac output was unchanged. With seizures associated with large elevations of arterial pressure, the central venous and left ventricular end-diastolic pressures were markedly increased indicating incipient congestive failure. The pressor response was blocked by alpha-adrenergic blockade with phentolamine. Increased regional vascular resistance was abolished by regional sympathectomy. While either adrenalectomy or treatment with 6-hydroxydopamine alone failed to abolish the pressor response, combined, they did. Such treatment unmasked an atropine-sensitive bradycardia. The pressor response with seizures is a consequence of increased vascular resistance in viscera and muscles due to widespread activation of sympathetic neurons and release of adrenomedullary catecholamines. Co-activation of cardiovagal and cardiosympathetic neurons may underlie some associated arrhythmias. Cardiovascular events may serve, by redistribution of the cardiac output, to assure increased availability of oxygen and nutrients to brain to meet the metabolic demands of convulsions.

INTRODUCTION

Epileptic seizures in man or animals are associated with a marked elevation of

the systemic arterial pressure^{2-4,7,18,27,28,32}. While it is known that during convulsions there is a substantial increase in cerebral blood flow^{2,27,28,32}, cerebral venous pressure²⁷ and an increase in resistance in the femoral arteries⁷, it has never been established if the elevation in arterial pressure is due to increased cardiac output or total peripheral resistance, and if the latter if the vasoconstriction observed in femoral arteries⁷ is widely distributed or only involves specific vascular beds. Such information is of interest for it raises the question as to whether the autonomic responses in epilepsy are generalized or involve selective and differentiated activation of the sympathetic nervous system^{10,33}.

In the present study we have therefore investigated in cat the changes in cardiodynamics and in the regional distribution of blood flow during convulsions produced electrically or by administration of convulsive agents.

METHODS

General

Twenty-five mongrel cats were anesthetized with ether. After insertion of cannulae in the femoral artery, femoral vein and trachea, each animal was paralyzed with gallamine triethiodide (5 mg/kg, i.v.), and artificially ventilated with 2% halothane in 100% O₂ delivered from a canister to a small animal respirator (Harvard Instrument Co.) via an anesthetic bag. The flow rate of halothane was adjusted so as to maintain adequate filling of the reservoir bag. End-tidal CO₂ recorded by an infrared gas analyzer (Beckman L1) was maintained at 2-3% throughout the experiment. The rectal temperature ranged from 36.5 to 38 °C, hypothermia being averted through use of a thermostatically regulated infrared lamp.

Recording of electroencephalogram (EEG)

The animal was placed in a stereotaxic frame and 3 small steel screws were placed in the skull. The first two screws were placed over the rostral portions of the parasagittal lobe on the surface of the dura mater and used as recording electrodes. The other screw was placed in the mid-portion of the occipital bone and served as an indifferent electrode. The EEG was recorded by an AC coupler (Beckman 9806A) with suitable amplification (100 μ V/cm) and displayed on a polygraph (Beckman Dynograph Recorder, 504A).

Measurement of cardiovascular functions

Systemic arterial pressure (BP) was recorded from a polyethylene catheter which was threaded up the femoral artery into the abdominal aorta and connected to a Statham P23Db transducer. The heart rate (HR) was computed from the blood pressure pulse by a cardi tachometer (Beckman 9857). End-tidal CO₂ was sampled through a fine catheter placed in a tracheal cannula, and was recorded by an infrared gas analyzer (Beckman LB-1). All cardiovascular activity was displayed on a polygraph.

Regional blood flow was recorded by a square-wave electromagnetic flowmeter

(Carolina Medical Electronics, types 322 and 332)⁸. Flow probes (Carolina Medical Electronics, Ep 403R, 404R, and 420R) were applied to the femoral, renal, mesenteric and common carotid arteries and the ascending aorta. No more than two probes were inserted in an animal at any one time. However, in any one experiment flow changes in numerous beds might be sampled. Renal flow was never measured after a laparotomy since the operation may produce reflex effect on renal flow¹⁶. After placement of the flow probe, the incision was closed and sutured areas were infiltrated with 2% procaine. Particular care was taken to assure mechanical stability of the arterial probe. The zero level of the flowmeter was established *in situ* before and after an experimental series by occluding the artery distally. At the termination of an experiment, the probes were calibrated by a standard method. In all instances mean blood flow was recorded through an integrated circuit built in the flowmeter.

The mean arterial pressure (Pm) was derived from the formula $(P_s + 2P_d)/3$, where P_s was systolic pressure and P_d was diastolic pressure⁸. Regional vascular resistance (RVR) was conventionally obtained from the formula: $RVR = P_m/F_m$, where F_m was mean blood flow in a given vascular bed⁸.

Cardiac output (CO) was estimated from the blood flow in the ascending aorta^{8,22}. Total peripheral resistance (TPR) was calculated from the formula: $TPR = (P_m - CVP)/CO$, where CVP was central venous pressure measured through a polyethylene catheter inserted into the right atrium through the femoral vein. Stroke volume (SV) was calculated from the formula: $SV = CO/HR$. Left ventricular end-diastolic pressure (LVEDP) was measured through a catheter threaded down the left common carotid artery and positioned in the left ventricle.

Induction of seizures

After catheters and flow probes were in place, the animal was taken from the stereotaxic frame and placed in a supine or recumbent position without restraint. The wound edges were infiltrated with procaine (2%) and lidocaine ointment (2%) was applied to the corneas. Halothane in 100% O₂ was then replaced by room air. During the remainder of the experiment paralysis was maintained by periodically administering gallamine, the trachea cleared and wounds reinjected with procaine. Any additional surgical procedures were performed after the animal was anesthetized with 2% halothane. With such care arterial pressure and heart rate remained at control levels during the duration of the experiment.

One hour after stopping halothane, seizures were induced by one of 4 different procedures. (1) Flurothyl ether (Indoklon) (0.05–0.075 ml) was injected by a small syringe through a hole in a tracheal cannula during the inspiratory phase of artificial ventilation. (2) Pentylenetetrazol (PTZ) (Metrazol) (10 mg/kg, i.v.) was injected into the femoral vein. (3) Transcerebral stimulation was performed by passing DC current (170 V, 100 mA, 1-sec pulse) from a device used for electroconvulsive therapy (Model B24, Medcraft Electronic Co.) through saline-soaked gauzes attached with a clip to the ears. (4) Direct cortical stimulation was performed through two thin monopolar electrodes consisting of a stainless steel wire (diameter 0.006 in.) coated with Teflon and bared at the tip for 1 mm and carried in a No. 28 stainless steel hypodermic needle.

The electrodes were placed bilaterally in the rostral portion of the marginal gyrus via small holes that were drilled through the parietal bone with a dental drill. These electrodes were fixed to the skull with dental acrylic cement. Seizures were induced by stimulation through both electrodes (cathodes) with a 10-sec train of square wave pulses of 0.5 msec duration at a stimulus frequency of 50 Hz, delivered from a pulse generator (Devices, Digitimer) through a constant current stimulus isolation unit (Devices MK IV). The stimulus current was 2–5 mA. The anode was a skin clip. In any one animal seizures could be produced repeatedly by the various methods described above following stimulus-free intervals of 10–15 min. The stimulus parameters used for electrical stimulation and the dose of convulsive agents were established in preliminary studies to predictably initiate a single seizure sustained for at least 20 sec (see Table I).

Experimental maneuvers

(1) *Autonomic blockade.* Alpha-adrenergic blockade was achieved by administration of phentolamine (1 mg/kg, i.v. CIBA), beta-adrenergic blockade by propranolol (1 mg/kg, i.v. Sigma Chemical Co.) and cholinergic blockade by atropine sulfate (0.2 mg/kg, i.v. Elkins-Sinn, Inc.).

(2) *Regional sympathetic denervation.* Denervation of the renal artery was accomplished by transection of the renal nerves via a retroperitoneal approach. The femoral artery was denervated by transection of both the sciatic and femoral nerves^{8,40}.

(3) *Bilateral adrenalectomy.* Both adrenals were removed through a laparotomy. Careful efforts were made to maintain homeostasis.

(4) *Chemical sympathectomy.* Acute chemical sympathectomy was performed by adaptation of the method of others^{9,13} by systemic administration of 6-hydroxydopamine (6-OHDA) hydrobromide (Regis Chemical Co.). In brief, each animal received 100 mg/i.v. divided into 3 equal doses of 33 mg. After each injection, heart rate and blood pressure were permitted to return to control levels before another injection was made, and at least 30 min was allowed to elapse between any two successive injections. After the first dose of 6-OHDA, 10 ml of 5% dextran was administered intravenously to prevent hemoconcentration¹³. The solution of 6-OHDA was prepared before injection by dissolving the drug in physiologic saline containing ascorbic acid (1 mg/ml) to block auto-oxidation. Seizures were not induced until at least 2 h after the first injection of 6-OHDA.

Tyramine hydrochloride (Sigma Chemical Co.) was used to assess the efficiency of chemical sympathectomy^{9,13}. The dose-response relationship was examined prior to and after administration of 6-OHDA with the graded injection of tyramine hydrochloride (20–200 μ g/kg, i.v.). After 2 h from the completion of the full dose of 6-OHDA (100 mg/animal, i.v.), the dose-response curve was shifted to the right with marked reduction both in amplitude ($P < 0.01$) and duration at all dosages.

Evaluation of data

For quantitative assessment of the changes in any cardiovascular parameter, the

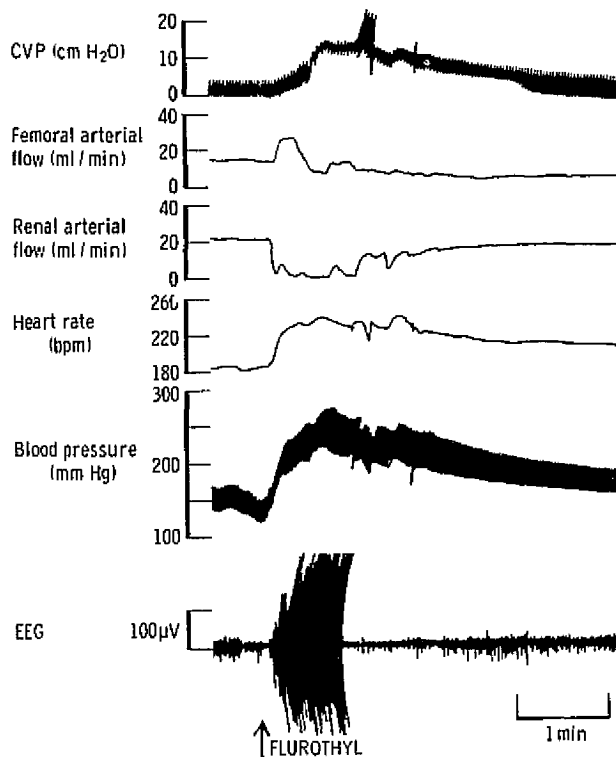


Fig. 1. Changes in arterial pressure, heart rate (in beats/min, bpm), blood flow in the renal and femoral arteries, and central venous pressure (CVP), during a convulsion produced by flurothyl ether in paralyzed artificially ventilated cat. See text for details.

responses during at least 3 representative seizures and pre-seizure control values were averaged for each animal. The means (control and seizure) were then used as a single value for that animal. Statistical evaluation was prefaced by comparing poststimulus to prestimulus value for the group, the n representing the number of animals, by use of a paired t -test³⁸. $P < 0.05$ were considered significant.

RESULTS

(I) Character of seizures

Seizures produced electrically by direct cortical or transcerebral stimulation or by the administration of the epileptogenic agents, flurothyl ether (Indoklon) or pentylenetetrazol (PTZ; Metrazol) were characterized by widespread spike and wave activity recorded over the cerebral cortex. The duration of the seizures evoked by the various methods in part differed. Seizures (as defined by the presence of spike and wave activity on the cortex) induced by direct cortical stimulation persisted the longest (59.5 ± 2.5 sec; $n = 18$); those initiated by transcerebral stimulation were the briefest

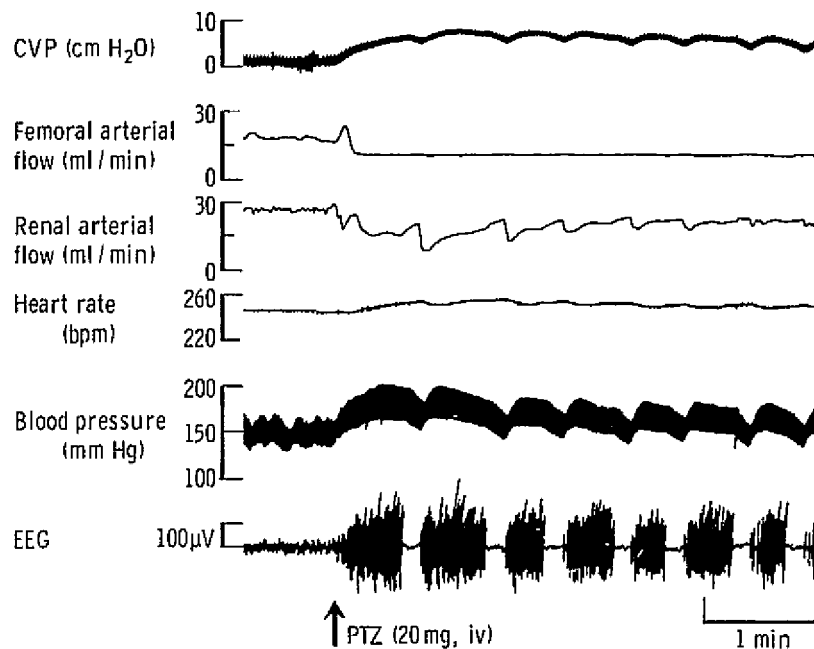


Fig. 2. Changes in arterial pressure, heart rate, blood flow in renal and femoral arteries, and central venous pressure (CVP) during a burst of repetitive seizures produced by pentyleneetetrazol (PTZ) (20 mg, i.v.) in paralyzed artificially ventilated cat.

(22.0 ± 1.0 ; $n = 14$) while the duration of seizures induced chemically by either fluoroethyl ether or PTZ lasted approximately 45 sec. In most instances the seizures occurred as a continuous uninterrupted burst of cortical high voltage activity (e.g., Fig. 1). In some instances, however, the seizures appeared in repetitive bursts lasting up to 1 min (Fig. 2).

Since the animals in this study were paralyzed, no motor manifestations of the seizure discharge were observed. However, all animals exhibited pupillary dilatation, retraction of the nictitating membrane, and profuse salivation in association with activation of the EEG.

(II) Cardiovascular changes with seizures

As previously noted^{2-5,7,16,27,28,32} seizures initiated by any means were associated with elevations in systolic, diastolic and mean arterial pressures (Figs. 1-7, Tables I and II). Sometimes, but not invariably, the rise of arterial pressure was preceded by a slight fall (Figs. 1 and 5). The onset of the alteration of arterial pressure began within the first 3 sec of the appearance of cortical electrical activity, peaked from 50 to 30 sec and remained elevated, sometimes for minutes, after the cortical EEG returned to normal. The magnitude of the elevation of arterial pressure varied with the mode of evoking the seizures. Under the conditions of this study transcerebral stimulation produced the largest elevation of mean arterial pressure (Table I) with peak elevations averaging 212 mm Hg. Chemical stimulation (Table I) increased

TABLE I

CHANGES IN CARDIOVASCULAR DYNAMICS DURING SEIZURES PRODUCED ELECTRICALLY (TRANSCEREBRAL) OR CHEMICALLY (FLUROTHYL ETHER) IN PARALYZED CAT

Values were measured at the time of maximum elevation of arterial pressure. n = number of animals. All values expressed as mean \pm S.E. of mean. Abbreviations: BP_m, mean arterial pressure; CVP, central venous pressure; CO, cardiac output; HR, heart rate; LVEDP, left ventricular end-diastolic pressure; SV, stroke volume. Significance ($P < 0.05$): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. ns = not significant. See Methods for method of statistical analysis.

	<i>Electrical stimulation (transcerebral)</i>				<i>Chemical stimulation (flurothyl ether)</i>			
	<i>Control</i>	<i>(n)</i>	<i>Seizure</i>	<i>% of control</i>	<i>Control</i>	<i>(n)</i>	<i>Seizure</i>	<i>% of control</i>
BP _m (mm Hg)	137 \pm 4	(14)	212 \pm 5	155***	128 \pm 2	(24)	198 \pm 3	155***
CVP (cm H ₂ O)	1 \pm 0	(13)	8 \pm 2	800***	2 \pm 0	(19)	7 \pm 1	350***
CO (ml/min)	459 \pm 13	(5)	491 \pm 36	107 ^{ns}	453 \pm 48	(5)	454 \pm 45	100 ^{ns}
TPR (mm Hg \cdot min/ml)	0.298 \pm 0.031	(5)	0.479 \pm 0.094	160***	0.289 \pm 0.054	(5)	0.440 \pm 0.054	152*
HR (beats/min)	235 \pm 3	(14)	219 \pm 5	93*	226 \pm 4	(24)	241 \pm 4	107*
SV (ml/beat)	2.0 \pm 0.1	(5)	2.1 \pm 0.5	105 ^{ns}	2.1 \pm 0.5	(5)	2.0 \pm 0.3	95 ^{ns}
LVEDP (mm Hg)	3 \pm 1	(5)	19 \pm 4	633**	4 \pm 1	(10)	11 \pm 2	275***
<i>Regional blood flow (ml/min)</i>								
Femoral artery	14.6 \pm 1.3	(13)	2.9 \pm 0.4	20***	16.2 \pm 1.3	(24)	19.3 \pm 2.7	119 ^{ns}
Renal artery	22.5 \pm 1.8	(11)	11.9 \pm 1.9	52***	26.8 \pm 2.0	(15)	11.1 \pm 2.2	41***
Mesenteric artery	22.8 \pm 3.1	(8)	10.7 \pm 2.3	47***	25.3 \pm 2.8	(13)	14.8 \pm 1.7	59**
Common carotid artery	39.0 \pm 2.0	(10)	79.4 \pm 5.5	203***	32.5 \pm 1.5	(8)	66.0 \pm 2.8	203***
<i>Regional vascular resistance (mm Hg \cdot min/ml)</i>								
Femoral artery	10.0 \pm 0.6	(13)	116.1 \pm 22.0	1161***	9.0 \pm 0.7	(24)	15.6 \pm 2.2	173**
Renal artery	5.9 \pm 0.3	(11)	24.7 \pm 5.0	419*	5.2 \pm 0.3	(15)	37.8 \pm 9.3	727**
Mesenteric artery	7.5 \pm 0.8	(8)	44.0 \pm 16.6	587*	5.8 \pm 0.5	(13)	16.2 \pm 2.0	279***
Common carotid artery	3.6 \pm 0.1	(10)	2.9 \pm 0.1	81*	3.9 \pm 0.1	(8)	2.8 \pm 0.2	72**

TABLE II

CHANGES IN CARDIOVASCULAR DYNAMICS DURING SEIZURES PRODUCED BY DIRECT CORTICAL STIMULATION IN CAT

Values were measured at the time of peak elevation of arterial pressure. All values, abbreviations and symbols are expressed as in Table I.

	<i>Transcortical stimulation</i>				
	<i>Control</i>	<i>(n)</i>	<i>Seizure</i>	<i>% of control</i>	
BP _m (mm Hg O)	121 ± 2	(24)	161 ± 4	133	***
CVP (cm H ₂ O)	3 ± 0	(19)	4 ± 1	133	ns
CO (ml/min)	452 ± 14	(5)	512 ± 20	113	*
TPR (mm Hg · min/ml)	0.293 ± 0.014	(5)	0.349 ± 0.018	119	*
HR (beats/min)	229 ± 5	(24)	247 ± 5	108	*
SV (ml/beat)	2.1 ± 0.1	(5)	2.3 ± 0.1	110	ns
LVEDP (mm Hg)	5 ± 1	(10)	3 ± 2	60	ns
<i>Regional blood flow (ml/min)</i>					
Femoral artery	16.0 ± 1.2	(18)	15.7 ± 1.4	98	ns
Renal artery	26.6 ± 1.6	(9)	28.7 ± 2.0	108	ns
Mesenteric artery	19.7 ± 0.5	(9)	12.4 ± 1.1	63	***
Common carotid artery	37.2 ± 1.4	(5)	65.2 ± 4.6	175	***
<i>Regional vascular resistance (mm Hg · min/ml)</i>					
Femoral artery	8.2 ± 0.5	(18)	11.8 ± 1.3	144	*
Renal artery	4.5 ± 0.1	(9)	5.5 ± 0.3	122	*
Mesenteric artery	6.4 ± 0.1	(9)	14.3 ± 1.2	223	***
Common carotid artery	3.6 ± 1.4	(5)	2.7 ± 0.1	75	***

Differs from control: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ns = not significant.

arterial pressure about as much. On the other hand, direct cortical stimulation (Table II) elicited peak elevations of mean arterial pressure which were substantially smaller than those initiated by other means. Since direct cortical stimulation produced the most prolonged seizures, it is evident that the duration of the seizure and the magnitude of the rise of arterial pressure do not correlate.

In general, the responses evoked by transcerebral stimulation or by the chemical agents were qualitatively similar and exhibited stereotyped and patterned circulatory responses, differing only in the magnitude of the component parts, and can be discussed as a group. In Table I the cardiovascular responses to transcerebral stimulation or, for brevity in response to one of the agents, flurothyl ether, are tabulated. On the other hand, the cardiovascular changes associated with direct cortical stimulation (Table II) were substantially different and will be discussed separately, below.

(III) Cardiovascular changes with seizures evoked electrically by transcerebral stimulation or chemically by convulsants

(A) Changes in cardiodynamics, total peripheral resistance, and central venous pressure

Seizures produced electrically by transcerebral shock, or chemically, were

associated with substantial elevations in the total peripheral resistance (Table I). Despite small individual and group variations in heart rate, never greater than 10% of control, the cardiac output was unchanged and stroke volume remained constant indicating that the increase in arterial pressure is entirely due to an increased peripheral resistance.

During the peak elevation of arterial pressure, left ventricular end-diastolic pressure was usually increased (Table I). Since cardiac output is maintained in the face of an elevation of total peripheral resistance during seizures it is probable that the force of myocardial contraction is also increased.

The central venous pressure was also substantially elevated during seizures (Figs. 1 and 2, Table I) rising 8-fold during seizures produced by transcerebral stimulation and 3-4-fold in chemically induced seizures (Table I). In addition, frequent bouts of ventricular arrhythmias were observed during the peak rise of arterial pressure in association with seizures produced by any means.

(B) Changes in regional blood flow and resistance

Rapid and marked changes in regional blood flow and resistances in the femoral, renal, mesenteric, and carotid arteries occurred during convulsions (Figs. 1 and 2; Table I). During seizures renal (Figs. 1 and 2) and mesenteric arterial blood flows were reduced by nearly 50% and regional resistances were substantially elevated. The changes in flow occurred within the first 5 sec of the onset of the seizure and persisted for many seconds following its cessation (Figs. 1 and 2). When seizures were repetitive (Fig. 2), reduction in regional flow might be relatively sustained throughout the ictal state.

Often, at the onset of seizures, there was a small and transient increase in femoral arterial flow (Figs. 1 and 2). Femoral blood flow then returned to control or even reduced levels in association with an increase in femoral arterial resistance, paralleling the elevation of systemic pressure. With electrically induced seizures, femoral arterial resistance increased 10-fold (Table I). With chemically induced seizures, changes of femoral resistance were less pronounced (Table I).

In contrast to the response in other vascular beds, blood flow increased and resistance decreased in the common carotid artery during seizures. These changes probably reflect the well established associated increase in cerebral blood flow^{2,27,28,32}.

(C) Peripheral mechanisms of cardiovascular changes with seizures

We next sought to establish the nature of the peripheral mechanisms mediating the elevation of arterial blood pressure and changes in regional blood flow and resistances associated with seizures.

(1) Adrenergic blockade. The systemic administration of the α -adrenergic antagonist phentolamine (1 mg/kg, i.v.) entirely blocked the elevation of arterial pressure and alterations in regional vascular resistances associated with seizures (Fig. 3). The β -adrenergic antagonist, propranolol (1 mg/kg, i.v.), had no such effect.

(2) Cholinergic blockade. Atropine sulfate administered systemically (0.2 mg/kg, i.v.) blocked the initial bradycardia which accompanied seizures induced by trans-

EFFECT OF PHENTOLAMINE ON PRESSOR RESPONSES TO SEIZURES

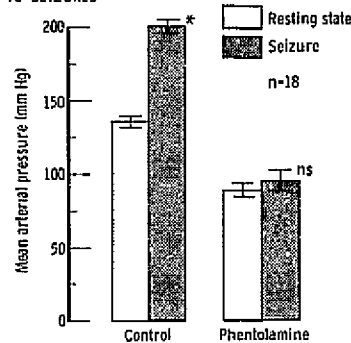


Fig. 3. Blockade of the pressor response during seizures by phentolamine (1 mg/kg, i.v.). Pooled data from 18 animals in whom seizures were produced by electrical stimulation.

cerebral stimulation. It did not affect the early rise in femoral arterial flow indicating that transient muscular vasodilatation was not due to activation of the cholinergic sympathetic muscular vasodilator system³⁹.

(3) *Regional sympathectomy.* The decrease in blood flow and increase in resistance in the femoral and renal arteries were abolished by surgical interruption of their sympathetic innervations. As illustrated in Fig. 4, interruption of the major sympathetic input to the hindlimb produced by transection of the femoral and sciatic nerves⁴⁰ entirely blocked the increase in femoral arterial resistance associated with a seizure resulting in the appearance of a passive increase in blood flow to the hindlimb. A similar reversal of decreased to increased blood flow with abolition of the seizure-induced increase in resistance occurred following sympathetic denervation of the renal artery.

(4) *Adrenalectomy.* Bilateral adrenalectomy did not alter the magnitude of the pressor response during seizures (Fig. 5). This finding indicates that a release of adrenomedullary catecholamines did not contribute to the rise in arterial pressure.

(5) *6-OHDA.* To further examine the contribution of sympathetic vasomotor neurons and the adrenal medulla to the elevated arterial pressure associated with seizure, cats were treated acutely with the adrenolytic agent 6-OHDA (see Methods). Following treatment, and at a time when sympathetic reactivity to tyramine is abolished, the arterial pressure and heart rate responses were profoundly altered, both quantitatively and qualitatively. As shown in a typical experiment (Fig. 6), and in pooled data (Fig. 7), treatment with 6-OHDA resulted in: (a) the appearance of an early fall in arterial pressure; (b) a reversion of a unimodal tachycardia, which began immediately with the onset of seizures, to a biphasic response of an initial bradycardia followed after a latency of approximately 15 sec by a sustained tachycardia; (c) the delayed appearance of an elevation of arterial pressure. The elevated pressure occurred approximately 20–30 sec after the seizures and persisted over 5 min contrasting with the control response (Fig. 6).

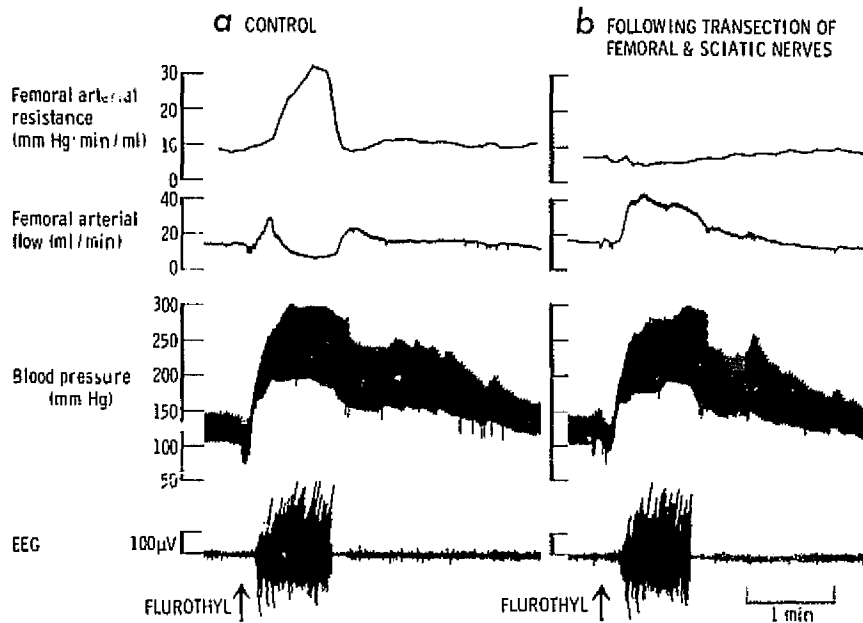
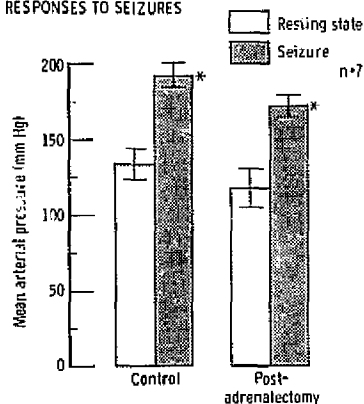


Fig. 4. Effects of local sympathetic denervation on changes in blood flow and resistance in the femoral artery during a convulsion produced by flurothyl ether (0.05 ml) in paralyzed and artificially ventilated cat. a: prior to denervation. Note brisk elevation in resistance and, following a small increase, a slight depression of blood flow in the femoral artery. b: 30 min following transection of femoral and sciatic nerves, which will effectively denervate the femoral artery^{8,10}. Note the absence of any increase in resistance and the passive increase in flow associated with a seizure producing a comparable elevation of arterial pressure as in a.

EFFECTS OF ADRENALECTOMY ON PRESSOR RESPONSES TO SEIZURES



* Differs from resting pressure ($p < .001$)

Fig. 5. Effect of adrenalectomy on resting mean arterial pressure and on the pressor response to seizures. Adrenalectomy was performed after establishing control values. Seizures were induced following termination of adrenal surgery. Note that adrenalectomy did not change the resting arterial pressure nor the magnitude of the pressor response.

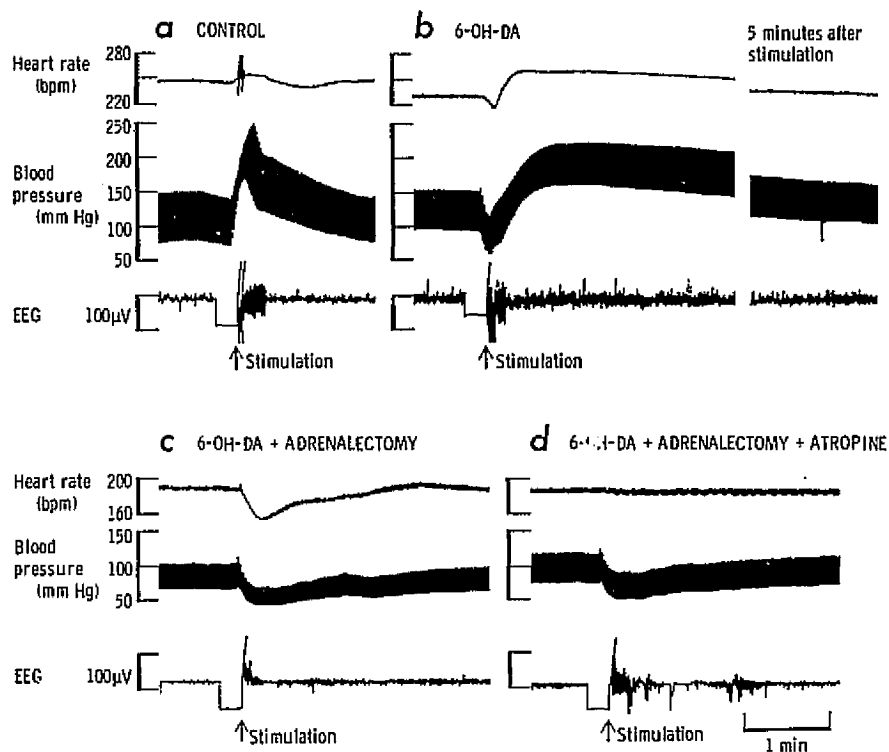
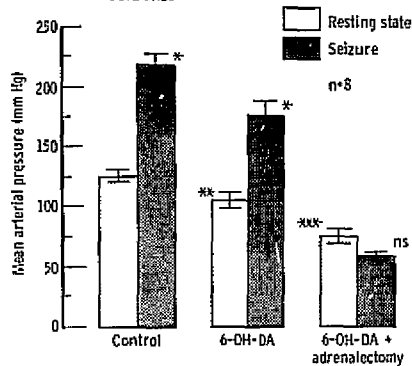


Fig. 6. Effects of 6-OHDA and adrenalectomy on the changes in arterial pressure and heart rate during seizures induced by transcerebral stimulation. a: control. b: following treatment with 6-OHDA. Note the fall of arterial pressure and heart rate at the onset of the seizures followed by a delayed and prolonged elevation in both responses. c: 6-OHDA and adrenalectomy. Note the onset of a depressor response and bradycardia with the seizures. d: effect of atropine (0.2 mg/kg, i.v.) administered 15 min prior to production of convulsions in adrenalectomized animal treated with 6-OHDA. Note the prolonged depressor response in the absence of bradycardia probably reflecting the reduction in cerebrovascular resistance.

The delayed elevation in arterial pressure and heart rate in 6-OHDA-treated animals was a consistent finding and was entirely abolished by adrenalectomy (Figs. 6 and 7), demonstrating that they were the result of the release of adrenomedullary catecholamines. Following 6-OHDA and adrenalectomy seizures were associated with a bradycardia and a *fall* of arterial pressure. The presence of bradycardia in the absence of a rise of arterial pressure indicates it was not due to baroreceptor reflex mechanisms. The fact that the bradycardia was abolished by atropine (Fig. 6) indicates that the bradycardia probably is the result of direct activation of central cardiovagal neurons and indicates that during seizures there is probably co-activation of sympathetic and vagal neurons to heart.

Despite treatment with 6-OHDA, adrenalectomy, and atropine, a fall in blood pressure sometimes occurred during seizures. While in some instances it might be marked (Fig. 6d) in the group as a whole it was small and not significant (Fig. 7). The mechanism of this hypotension is unknown. Its persistence after blockade of the

EFFECTS OF 6-OH-DA AND ADRENALECTOMY ON PRESSOR RESPONSES TO SEIZURES



Differs from resting state: * $p < .001$
 Differs from control: ** $p < .05$, *** $p < .01$
 ns = not significant

Fig. 7. Effects of 6-OHDA and adrenalectomy on resting mean arterial pressure and the pressor response to seizures. Note that although 6-OHDA depressed the resting arterial pressure it did not significantly attenuate the pressor response. Following 6-OHDA and adrenalectomy the resting pressure declined further and the pressor response to seizures was abolished. See text for details.

bradycardia with atropine indicates the response is not due to change in cardiac output, and its persistence in the presumed absence of sympathetic nerves and adrenal medulla indicates it is not a consequence of sympatho-adrenal inhibition or release of vasodilators from sympathetic nerves. Since the animals were treated with gallamine a significant role of cholinergic release of muscle movement is unlikely. One plausible explanation is that it is due to the fall in cerebral vascular resistance secondary to the seizure.

(IV) Cardiovascular changes with seizures produced by direct cortical stimulation

Seizures produced by direct cortical stimulation, although having the longest duration, elicited the smallest elevation of arterial pressure (Table II) in comparison to seizures elicited by transcerebral stimulation or by convulsants (Table I). In addition to the attenuated rise of arterial pressure, seizures evoked by direct cortical stimulation were associated with an elevation of cardiac output and of total peripheral resistance indicating that both mechanisms contribute to the rise of arterial pressure. In addition, seizures produced by cortical stimulation were characterized by an absence of changes in left ventricular end-diastolic and central venous pressures. However, as with the other types of seizures, there was an increase in resistance in the femoral renal and, particularly, the mesenteric arteries. Reciprocally, there was an increase in blood flow and decrease in resistance in the common carotid artery.

DISCUSSION

The present study demonstrates that generalized seizures evoked either by electrical stimulation or by the epileptogenic agent flurothyl ether or PTZ in paralyz-

and artificially ventilated cats is associated with profound and stereotyped changes in cardiovascular function. As noted by others, the major change is an abrupt elevation of arterial pressure (sometimes preceded by a transient fall) which long outlasts the seizure. The rise of pressure is exclusively due to an increase in total peripheral resistance when the seizures are elicited by transcerebral or chemical stimulation and reflects an increase in resistance and sometimes a decrease in blood flow in the femoral, renal and mesenteric arteries. Cardiac output and stroke volume are unchanged during seizures produced by direct cortical stimulation, in which the increase of total peripheral resistance and hence of arterial pressure and ventricular overload are reduced. There was, in addition, a small rise of cardiac output.

The increase in peripheral resistance in seizures is produced by excitation of sympathetic preganglionic vasomotor neurons and mediated by α -adrenergic receptors. Surgical denervation or pharmacological blockade with the α -adrenergic blocking agent phentolamine, but not the β -blocking agent, propranolol nor the cholinergic blocker atropine, abolished the pressor responses as well as concomitant changes in flow and resistance in renal, mesenteric and femoral arteries. During seizures, however, there is also a parallel release of adrenomedullary catecholamines. While the release of these amines does not, in the presence of intact vascular innervation, contribute to the cardiovascular events, when vasomotor nerves are destroyed by treatment with 6-OHDA the amount released is sufficient to elevate arterial pressure. The release of adrenal catecholamines during seizures probably does not contribute significantly to cardiovascular homeostasis. The catecholamines may, however, serve a role in regulating, phasically, the metabolism of myocardium and skeletal muscle, possibly to compensate for the sudden increased metabolic demands associated with seizure activity in the unanesthetized state.

Seizures produced by transcerebral stimulation, and to a lesser degree chemically, were associated with a marked elevation of central venous pressure. The increase of venous pressure was not only due to a sudden increase in left ventricular pressure since in some instances, particularly with PTZ, a 3-4-fold rise of venous pressure occurred unassociated with any elevation of left ventricular pressure. In these instances the increase in central venous pressure probably reflects peripheral venoconstriction. However, during seizures produced by transcerebral stimulation left ventricular end-diastolic pressure was increased and central venous pressure was increased up to 8-fold. In this circumstance it is conceivable that some of the increase in venous pressure was due to ventricular failure. Thus, when the cardiovascular responses are intense, seizures may be accompanied by incipient congestive failure.

In contrast to the stereotyped changes in arterial pressure and peripheral resistance associated with seizures, the direction of change in heart rate was variable. The variability of the cardiac rate responses in individual groups was probably a result of opposing neural influences tending either to accelerate (*e.g.* direct sympathetic stimulation or the release of adrenomedullary catecholamine) or to slow it (*e.g.* direct excitation of cardiovagal fibers or reflex vagal activation by baroreceptor mechanisms). In addition, the conditions of the experiment including the use of the paralytic agent gallamine, which has a mild atropine-like action^{23,34,35}, and artificial ventilation,

which will modify ventricular filling, may also have contributed to the variability of the cardiac rate responses. In general, it appeared that sympathetic excitation of the heart often occurred; however, when the arterial pressure was substantially elevated, for example, with seizures induced by transcerebral stimulation, the baroreceptor mechanisms might intervene to produce slowing.

Of particular interest was the observation in experiments with 6-OHDA that during seizures there was a co-activation of the cardiac vagus and sympathetic fibers to the heart. Such dual activation of cardiac sympathetic and vagus nerves when elicited by electrical stimulation centrally or peripherally, as Manning and Cotten²⁵ have demonstrated, creates conditions which favor the development of cardiac arrhythmias. This fact may be the basis for the arrhythmias which we observed to be associated with the maximal rise of blood pressure during seizure activity and also those which on occasion may be seen during electroconvulsive therapy in man²⁶.

At a time when arterial pressure and resistance in peripheral vessels were increased, there was a significant elevation of blood flow and reduced resistance in the common carotid artery. The pressor response by itself cannot explain the rise in carotid arterial blood flow. Elevation of the arterial pressures to a comparable magnitude by pressor agents^{20,21,30} does not increase flow and reduce resistance in the carotid arteries because of autoregulation⁴³. However, when cerebral autoregulation is impaired, blood flow rises passively during systemic arterial hypertension^{29,32}. The fact that during seizures cerebral vascular autoregulation is suspended, possibly as a consequence of accumulation of local metabolites¹⁷, therefore, is the most probable explanation for the increased carotid blood flow observed in this study.

The studies reported here and the work of others allow the following formulation for the circulatory changes which occur during seizures. (a) At the onset of a seizure, there is a marked increase in the discharge of peripheral sympathetic neurons resulting in a marked increase in vascular resistance in viscera and limbs, in venoconstriction, and in the release of adrenal medullary catecholamines. (b) The increase in systemic vascular resistance results in a rise in arterial pressure accounting for the pressor response. If powerful enough, it may counterbalance the increase in myocardial contraction and heart rate and thereby counteract a potential elevation in cardiac output. However, if the elevation of peripheral resistance is small some elevation of cardiac output may occur. (c) When the activation of the peripheral sympathetic neurons is marked, there may be a rise in left ventricular end-diastolic pressure and an increase in circulating blood volume resulting in elevated central venous pressure. (d) Concurrently, there is a parallel suspension of autoregulation of the cerebral blood flow probably due to the accumulation of acid metabolites in brain resulting thereby in the elevation of cerebral blood flow. The net result of these events is to mobilize the amount of blood perfusing brain to meet the increased metabolic demands of the seizure as well as to compensate for the transient hypoxemia produced by the apnea or hypoventilation associated with major motor seizures in the natural (*i.e.* unparalyzed) state.

The general patterning of the changes in cardiovascular activity and associated pupillary dilatation, retraction of the nictitating membrane, and salivation implies

that during seizures there is a widespread and undifferentiated activation of the sympathetic nervous system. Those end organ responses which differ from what would be predicted by generalized sympathetic activation (as for example the variable changes in heart rate and the decrease in carotid arterial resistance) can be attributed, as pointed out above, to either an interaction with secondary reflex effects or an overriding local autoregulation. The circulatory pattern in seizures therefore must be distinguished from the highly differentiated pattern of sympathetic discharge leading to a combination of altered cardiodynamics and redistribution of blood flow associated with such behaviors as feeding^{12,36}, sleep^{14,22}, defence^{1,11,24}, or diving^{1,42}. Indeed, the autonomic response during seizures may be the only clearly demonstrated example of reflexly elicited mass excitation of the sympatho-adrenal axis which, at one time, was always believed to discharge as a unit⁶.

The differences in the magnitude of the pressor response to different modes of stimulation is of interest. The most potent increase in arterial pressure and, in general, other cardiovascular events, occurred with transcerebral stimulation; the least with direct cortical stimulation. Thus, despite equivalent activation of the neocortex, the autonomic changes were reduced with direct stimulation. It is evident, therefore, that discharge in neocortex alone is not the driving force behind most of the activation of the autonomic nervous system with seizures. Rather it must require activation of either deeper areas of the forebrain, probably in the limbic system, and/or sites within the upper brain stem. The nature of these networks remains to be determined. However, the fact that the induction of seizures by either of the epileptogenic agents utilized in this study (flurothyl ether or PTZ) produced cardiovascular changes almost equivalent to those of transcerebral stimulation indicates that these agents must also act upon the critical areas in ways equivalent to the transcerebral electrical stimulus.

The clinical implications of these ictal circulatory changes are uncertain. Thus, despite the impressive elevation in arterial pressure which occurs with seizures and the possibility of cardiac overload, cardiovascular complications of seizures in man are apparently uncommon^{19,31}. Most such complications have been reported in relation to electroshock therapy. Cerebrovascular complications are similarly rare³¹, although one might surmise that systemic hypertension and an abrupt rise in central blood flow would favor intracranial hemorrhage in predisposed subjects (*e.g.* those with occult aneurysms, arteriovenous malformations, or advanced atheromatous disease). Renal injuries following repeated seizures have occurred, both in patients with epileptogenic intracranial lesions and in those receiving electroshock therapy³⁷. These could be the result of renal ischemia^{15,41}, our studies indicating an evident fall in renal artery flow with induced seizures. In any event, the cardiovascular status of those undergoing electroconvulsive therapy and those experiencing repetitive seizures merits careful attention.

ACKNOWLEDGEMENTS

Supported by grants from NIH (NS 08476 and NS 3346) and NASA (NGR 33010-179).

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Changes in Cyclic Nucleotide Metabolism in Aorta and Heart of Neurogenically Hypertensive Rats: Possible Trigger Mechanism of Hypertension

(neurogenic hypertension/adenylate cyclase sensitivity/sympathetic role in hypertension/3':5'-cyclic-AMP and 3':5'-cyclic-GMP phosphodiesterases)

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Communicated by Bernard B. Brodie, March 7, 1975

ABSTRACT Changes in cyclic nucleotide metabolism similar to those characteristic of the chronic forms of hypertension were observed in an acute neurogenic form of hypertension in rats produced by electrolytic lesions of the *nucleus tractus solitarii*. These changes that were evident 2 hr after the lesions were made included decreased cyclic AMP levels in the heart, increased cGMP:cAMP ratio, cAMP phosphodiesterase (3':5'-cAMP 5'-nucleotidohydrolase, EC 3.1.4.17) and guanylyl cyclase [GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2] activities in the aorta and decreased sensitivity of adenylyl cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] in both the aorta and heart to stimulation by the β -adrenergic stimulant isoproterenol. These changes appear to depend on catecholamine release and are not due to mechanical distortion secondary to the increased arterial pressure. These studies provide biochemical support to the concept that the sympathetic nervous system may play a critical role in the initiation of the hypertensive syndrome and that chronic hypertension could result from the fixation of the biochemical effects of increased sympathetic activity.

There is some evidence that the sympathetic nervous system has a critical role in initiating or sustaining essential hypertension in man (1, 2). How augmented sympathetic nerve activity can produce a fixed arterial hypertension is not known. One view (3, 4) suggests that sustained states of increased sympathetic nerve activity, perhaps caused by heightened states of emotion, can lead to structural vascular changes, which, by reducing the wall-lumen ratio, could result in increased vascular resistance and serve to "fix" the hypertension. Thus, resumption of sympathetic discharge to normal levels would not reverse the increased vascular resistance and the elevated arterial pressure. Transformation of a transient to a fixed state of vascular resistance might thereby serve as a link between neurogenic and essential forms of hypertension. In accord with this view, prolonged arterial hypertension has been elicited in several animal species by emotional or physical stress (5-7), by conditioning (8), or by repetitive electrical stimulation of the brain (9).

The mechanisms through which the activity of sympathetic impulses mediates changes in the arterial walls are not understood. One mechanism could be changes in the metabolism of cyclic nucleotides in the blood vessel wall. The cyclic nucleotide system might, therefore, represent the interface

between the sympathetic nerves and blood vessels, particularly since the levels of adenosine 3':5'-cyclic monophosphate (cAMP) and guanosine 3':5'-cyclic monophosphate (cGMP) appear to play a critical role in determining the tone of smooth muscles (10, 11). Moreover, the metabolism and accumulation of these cyclic nucleotides can be altered by catecholamine neurotransmitters (12). Changes in the metabolism of these important messengers have already been shown to characterize the vessels and heart from animals with three chronic forms of hypertension in rats (13, 14). In the present study we therefore sought to determine if changes in cyclic nucleotide metabolism, similar to those seen in chronic states of arterial hypertension, could be produced in an acute hypertensive state that is clearly due to elevated sympathetic nerve activity. This form of neurogenic hypertension, discovered recently by Doba and Reis (15, 16), results from central deafferentation of baroreceptors produced by placement of bilateral electrolytic lesions within the *nucleus tractus solitarii* (NTS) in the brainstem.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Carworth Farms, New York) weighing 300-400 g were used in this study.

The production of arterial hypertension by bilateral lesions of NTS has been described in detail elsewhere (15). In brief, the rats were anesthetized with halothane (3% in 100% O₂ blown over the nose through a face mask). An arterial cannula was inserted in either the femoral or ventral tail artery and the region of the obex was exposed by an occipital craniotomy. A Teflon-coated electrode, with an exposed tip of 0.3 mm and carried by a micromanipulator, was placed into the NTS at the level of the obex. Electrolytic lesions were made by passing a dc current, the wounds were closed, and the animals were permitted to recover from anesthesia. Sham-operated controls were treated similarly but without placing the electrodes in the brain. In some animals, the blood pressure was monitored continuously. After 2 hr, the animals were killed by decapitation and the hearts and the aortas (from heart to femoral bifurcation) were rapidly removed. A portion of each tissue was rapidly frozen in liquid nitrogen and kept frozen at -20° until analyzed for cyclic nucleotide contents, not more than 2 weeks later. The rest of the fresh tissue was used for the enzyme assays; two to three aortas were pooled for most assays. In some experiments, designed to examine the effects of elevated blood pressure *per se* on cyclic nucleotide metabolism in the heart, the aorta was ligated just below the dia-

Abbreviations: cAMP, adenosine 3':5'-cyclic monophosphate; cGMP, guanosine 3':5'-cyclic monophosphate; NTS, *nucleus tractus solitarii*.

TABLE 1. Changes in cyclic nucleotide metabolism in aortas and hearts of rats with acute neurogenic hypertension

	Control		Hypertensive	
	Aorta	Heart	Aorta	Heart
Cyclic nucleotide contents*				
cAMP	0.38 ± 0.05	0.66 ± 0.08	0.18 ± 0.01†	0.47 ± 0.04†
cGMP	0.09 ± 0.03	0.06 ± 0.01	0.15 ± 0.03†	0.05 ± 0.01
Nucleotide "index"‡	—	—	1.76§	1.17
Adenylyl cyclase activity¶				
Basal	9.95 ± 1.12	33.00 ± 0.47	7.04 ± 0.52	29.79 ± 0.99
Isoproterenol	12.32 ± 1.23	38.55 ± 2.38	7.00 ± 0.42	28.67 ± 3.08
Sodium fluoride	14.67 ± 2.23	42.47 ± 2.64	11.86 ± 1.12	35.78 ± 1.83
Guanylyl cyclase activity¶				
Basal	0.99 ± 0.24	4.75 ± 0.23	1.90 ± 0.25†	5.63 ± 0.46
Phosphodiesterase activity**				
cAMP as substrate:				
Total activity	11.5 ± 1.5	60.8 ± 11.8	15.8 ± 4.5	62.7 ± 12.7
% of low K _m ††	2.6	2.2	3.8†	2.9†
cGMP as substrate:				
Total activity	12.7 ± 1.1	87.1 ± 2.0	13.6 ± 1.3	81.8 ± 4.0
% of low K _m ††	5.4	2.1	4.7	2.3
Mean aortic blood pressure‡‡	122.5 ± 2.1		172.6 ± 7.0§§	

* pmol/mg wet tissue; mean of four to ten determinations ± SEM.

† Significantly different from control ($P < 0.05$).‡ $\frac{\text{cAMP control}}{\text{cGMP control}} \div \frac{\text{cAMP hypertensive}}{\text{cGMP hypertensive}}$ § Significantly different from 1 ($P < 0.05$).

¶ pmol of cyclic nucleotide formed/mg wet tissue per min; mean of four to ten determinations ± SEM.

|| Significantly different from basal ($P < 0.05$).

** nmol cyclic nucleotide hydrolyzed/5 mg wet tissue per 10 min at 30°; mean of four to ten determinations ± SEM.

†† Calculated as described previously (13) from the data obtained at 15 substrate concentrations.

‡‡ mm Hg; (1 mm Hg = 133 Pa) mean of six determinations ± SEM.

§§ Significantly different from control ($P < 0.01$).

phragm and the adrenal glands and kidneys were removed. We carried out the latter procedure to eliminate the release of pressor agents from the ischemic organs. Aortic ligation elevated the blood pressure in the vessels preceding the ligation. The adrenals and kidneys were also removed from the corresponding controls but the aorta was not ligated. Blood pressure was measured in some of these animals through a cannula placed in the carotid artery.

cAMP, cGMP, cAMP phosphodiesterase (3':5'-cAMP nucleotidohydrolase, EC 3.1.4.17), adenylyl cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] and guanylyl cyclase [GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2] were assayed by the methods described earlier (14). The cyclic nucleotide index was calculated as follows:

Index =

$$\frac{\text{cAMP level in control}}{\text{cGMP level in control}} \div \frac{\text{cAMP level in hypertensive}}{\text{cGMP level in hypertensive}}$$

This index would reflect relative changes in the levels of either cyclic nucleotide with respect to the other.

RESULTS

As previously described (15, 16), bilateral electrolytic lesions in the NTS region of rat brainstem produced a considerable rise in pressure that was still sustained 2 hr later. The lesions also elicited significant changes in the amounts of cyclic nucleotides within the aorta and heart (Table 1). The level

of cAMP was reduced in both organs, whereas the level of cGMP was increased in the aorta, but not in the heart.

The ratios of cGMP to cAMP were markedly elevated in the aorta of animals with lesions, with only a very small change in the ratio within the heart. This fact is evident in the elevated cyclic nucleotide index, which reflects changes in cAMP to cGMP ratios in the treated animals as related to the sham controls. A cyclic nucleotide index higher than 1 indicates that, in the tissues from the animals with lesions, the levels of cGMP were relatively higher than the cAMP levels, compared to the tissues of the sham controls. The basal levels of adenylyl cyclase in animals with lesions did not differ from that in controls. However, the sensitivity of adenylyl cyclase to activation by the β -adrenergic agonist isoproterenol, but not sodium fluoride, was impaired. In addition, the basal activity of guanylyl cyclase was elevated in the aorta but not in the heart.

No significant changes were found in the total phosphodiesterase activity in animals with NTS lesions when compared to controls irrespective of the substrate used (Table 1). However, the amount of the low- K_m (high-affinity) form of the enzyme [which is probably the more important form biologically (13)] was significantly increased in both the aortas and hearts of animals with NTS lesions, when cAMP was used as a substrate. These findings demonstrate, therefore, that there probably is increased degradation of cAMP, but not cGMP, in both organs.

TABLE 2. Effect of systemic 6-hydroxydopamine and adrenalectomy on cyclic nucleotide levels* in aorta and heart and systemic blood pressure

	6-OH-dopamine + adrenalectomy		6-OH-dopamine + adrenalectomy + NTS lesion	
	Aorta	Heart	Aorta	Heart
Cyclic nucleotide contents				
cAMP	0.37 ± 0.04	0.61 ± 0.04	0.39 ± 0.04	0.76 ± 0.10
cGMP	0.05 ± 0.01	0.07 ± 0.01	0.05 ± 0.02	0.07 ± 0.02
Nucleotide "index"†	—	—	1.07	1.24
Mean aortic blood pressure‡	80 ± 8		76 ± 8 (n = 12)	

Rats received 6-OH-dopamine (100 mg/rat, intravenously); 24 hr later, under halothane anesthesia, the adrenal glands were removed bilaterally and lesions were placed in NTS of the experimental group. Sham-operated animals only received the drug and had adrenal glands removed. Two hours later the animals were killed and aortas and hearts were removed.

* pmol/mg wet tissue; average of four to ten determinations ± SEM.

† $\frac{\text{cAMP control}}{\text{cGMP control}} \div \frac{\text{cAMP hypertensive}}{\text{cGMP hypertensive}}$

‡ mm Hg; average of six determinations ± SEM.

Systemic 6-OH-dopamine and adrenalectomy entirely prevented the rise of blood pressure produced by NTS lesions as was previously described (16) (Table 2). This treatment also blocked any changes in the levels of cAMP or cGMP in aorta or heart.

Aortic ligation elevated the blood pressure in the carotid artery to levels close to those seen in NTS hypertension (Table 3). Aortic ligation, however, not only failed to produce a decrease in myocardial cAMP, but indeed elicited the reverse changes in both cAMP levels and phosphodiesterase activity in the heart; i.e., cAMP levels were higher, while the percentage of high-affinity phosphodiesterase activity was reduced. Furthermore, total phosphodiesterase activity was higher and the percentage of high-affinity phosphodiesterase was lower when cGMP was used as substrate.

DISCUSSION

The present study demonstrates that an acute increase in sympathetic discharge is capable of producing alterations in cyclic nucleotide metabolism similar to those found in chronic forms of hypertension (14). The prominent changes are a decreased concentration of cAMP in the aorta and the heart and an increased concentration of cGMP in the aorta. The changes occur within 2 hr after the development of increased sympathetic activity elicited by the NTS lesion. Presumably, they are due to an increased release of catecholamines.

The decrease in cAMP in both aorta and heart appears to result from two concurrent processes. The first and most important is an increased activity of the high-affinity phosphodiesterase, an effect which would accelerate the degradation of cAMP. The second process is the decreased sensitivity of adenylyl cyclase to activation, as shown by the strongly reduced effects of the β -adrenergic agonist isoproterenol. Since the basal activity of this enzyme did not change, it is unlikely to be responsible for the lower cAMP levels after the NTS lesion. The incapacity of this enzyme to be activated by adrenergic stimulation, as shown by its lower sensitivity to isoproterenol, suggests that the capacity of the system to increase cyclic AMP synthesis during sympathetic stimulation

is impaired. This would result in an imbalance in cyclic nucleotide synthesis in favor of GMP.

The decreased sensitivity in adenylyl cyclase to stimulation is also characteristic of diseases other than hypertension and may play an important role in their etiology (17). Loss of adenylyl cyclase sensitivity was observed in *in vitro* systems after excessive stimulation (18). The present studies may pro-

TABLE 3. Effects of aortic ligation on cyclic nucleotide metabolism in heart

	Control	Ligation
Cyclic nucleotide contents*		
cAMP	0.63 ± 0.05	0.89 ± 0.08†
cGMP	0.05 ± 0.01	0.06 ± 0.01
Nucleotide "index"‡	—	0.85
Phosphodiesterase activity§		
cAMP as substrate:		
Total activity	35.8 ± 2.3	62.0 ± 3.7†
% of low K_m ¶	3.2	1.76†
cGMP as substrate:		
Total activity	51.7 ± 3.8	106.7 ± 10.2†
% of low K_m ¶	4.3	2.2†
Mean carotid blood pressure	118.0 ± 4.0	168.0 ± 8.0**

The aortas were ligated just below the diaphragm under halothane anesthesia and the adrenals and kidneys were removed. In the controls, laparotomy was performed and adrenals and kidneys were removed without ligating the aorta. Blood pressure was measured by direct cannulation of the carotid artery 2 hr after surgery. Immediately following the blood pressure measurements, the animals were killed and the hearts were removed.

* pmol/mg wet tissue; average of seven determinations ± SEM.

† Significantly different from control ($P < 0.05$).

‡ $\frac{\text{cAMP control}}{\text{cGMP control}} \div \frac{\text{cAMP hypertensive}}{\text{cGMP hypertensive}}$

§ nmol cyclic nucleotide hydrolyzed per 15 mg wet tissue 10 min at 30°; mean of seven determinations ± SEM.

¶ Calculated as described previously (24) from the data obtained at 15 substrate concentrations.

|| mm Hg; mean of six determinations ± SEM.

** Significantly different from control ($P < 0.01$).

vide an *in vivo* confirmation of these *in vitro* systems, since the loss of sensitivity results from excessive sympathetic discharge and the associated increased concentrations of norepinephrine released at the nerve endings.

In the aorta, but not the heart, the decrease in cAMP levels is paralleled by an increase in the quantity of cGMP. The increased levels of cGMP appear primarily to be related to an increase in the synthesis of this nucleotide, since the only change in its metabolism is an increase in the activity of guanylyl cyclase without any changes in the degradation of the cGMP by phosphodiesterase. Thus, in the aorta there was increased degradation of cAMP coupled with increased synthesis of cGMP.

The changes in cyclic nucleotide levels appear to be related to the increase in discharge of the sympathetic nervous system elicited by the brainstem lesion and not to the elevated blood pressure *per se*. The evidence for this conclusion is 2-fold. First, systemic administration of 6-OH-dopamine combined with adrenalectomy not only prevents the elevation of blood pressure produced by the lesion (16), but also blocks the lesion-induced changes in cyclic nucleotide metabolism. This dose of 6-OH-dopamine will reduce the concentrations of norepinephrine in the myocardium (16) and functionally denervate peripheral vessels, making them unresponsive to sympathetic nerve stimulation (19, 20). The observation also demonstrates that the acute changes in cyclic nucleotide metabolism in the aorta and heart, which follow NTS lesions, are related to the release of catecholamines from sympathetic nerves and possibly the adrenal medulla.

Second, elevation of the intravascular pressure in vessels of the upper body and within the heart by acute ligation of the abdominal aorta failed to produce reduction in the concentrations of cAMP in the heart. Indeed, aortic ligation and the consequent increase in blood pressure in the chest resulted in an increase in cAMP. Since elevation of arterial pressure produced by aortic ligation will increase baroreceptor reflex activity and hence reduce the sympathetic drive to the heart, the findings suggest that the levels of this nucleotide are in a dynamic state, increasing or decreasing in response to fluctuations of the level of sympathetic nerve activity. These findings would parallel the observations of Deguchi and Axelrod on the regulation of the pineal gland by dynamic changes in sympathetic nerve activity (21).

The rise in cGMP levels in the aorta that is brought about by the increase of guanylyl cyclase activity may also reflect the increase of adrenergic tone which is apparently present in these animals. cGMP production is known to increase in human plasma (22) and platelets (10), rat vas deferens (23), and rabbit gallbladder (24) after α -adrenergic stimulation.

It is well recognized that the disease of essential hypertension in man is primarily due to increased peripheral resistance, primarily involving the smaller peripheral arteries and arterioles. Studies with more peripheral vascular beds would be more meaningful than studies with the aorta. In an earlier study (14), the changes in cyclic nucleotide metabolism in spontaneously hypertensive rats were similar in both the aorta and mesenteric artery. It may, therefore, be inferred that in the NTS-lesion-induced hypertension, changes in cyclic nucleotide metabolism similar to those already described for the aorta exist in the more peripheral vascular beds.

In conclusion, the study demonstrates that a 2-hr increase in sympathetic nerve discharge produced by brain lesions and

resulting in hypertension will elicit pronounced changes in the amounts and metabolism of cyclic nucleotides in the heart and aorta. The profile of these changes is similar to those seen in the same organs of rats in which hypertension has been produced chronically either as a consequence of a genetic defect (the spontaneously hypertensive rat), increased sodium intake, or prolonged physical and emotional stress (14). It is not known whether the changes in cyclic nucleotide metabolism observed are reversible. However, the possibility exists that these changes may become irreversible on repeated exposure to elevated sympathetic tone. Our findings therefore suggest that, when sustained, the neurogenically mediated biochemical changes in the circulatory system may become "fixed," producing an increased state of resistivity that characterizes some states of hypertension in man and animals (4). These sympathetically mediated changes in blood vessels may serve as a link between the transient and reversible elevations of blood pressure that characterize short periods of emotional arousal and the more enduring elevation of blood pressure characterizing the disease of essential hypertension.

The authors wish to acknowledge the competent technical assistance provided by Cindy Forman, Carol Gatewood, and Gary Gentry.

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Two specific brainstem systems which regulate the blood pressure

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SUMMARY

1. Two systems of brainstem involved in the regulation of arterial pressure, the nucleus solitarii (NTS) and a dorsal region of the pontomedullary tegmentum have been objects of investigation in our laboratory.

2. The NTS mediates baroreceptor reflexes in brain. Lesions in rat result in development of fulminating arterial hypertension as a consequence of abolition of baroreceptor reflexes centrally and release of sympathetic vasomotor neurons from inhibition.

3. In cat, NTS lesions result in the development over 1 week of labile neurogenic hypertension which is sustained.

4. Neuronal systems of the upper brainstem as well as neuronal systems which store, synthesize and release the catecholamine neurotransmitters are involved in NTS-mediated regulation of the BP.

5. A pressor system in the pontomedullary tegmentum consists of neurones which respond to focal distortion and mediate the pressor response to increased intracranial pressure (the Cushing reflex).

6. Lesions transecting the caudal pathway of the tegmental pressor system results in a fall of blood pressure to levels resulting from transection of the spinal cord.

7. This tegmental pressor system may correspond to the tonic vasomotor neuronal system of the lower brainstem whose integrity is necessary for the maintenance of normal levels of blood pressure.

Key words: baroreceptor reflexes, blood pressure, brainstem, brain catecholamines, intracranial pressure, reticular formation, solitary nucleus.

INTRODUCTION

It has long been recognized that the regulation of arterial pressure (BP) depends upon the activity of numerous neuronal systems in the central nervous system (CNS). These may be broadly divided into two groups on the basis of their opposing actions on the discharge of

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sympathetic neurones. The first group includes systems which facilitate sympathetic discharge; collectively they may be termed pressor systems; the second group consist of systems which inhibit sympathetic discharge and are termed depressor systems. Both pressor and depressor systems may act briefly (i.e. phasically), or may exert a continuous (i.e. tonic) effect on the BP. The maintenance of natural levels of BP and the appropriate reactivity of the circulation depends upon a balanced interaction between these systems. However, perturbations of central neural integration might lead to arterial hypertension if conditions result in augmented sympathetic firing effected either by augmentation of the discharge of pressor systems or by withdrawal of depressor input (*cf.* Reis & Doba, 1974).

Recent studies in our laboratory have been directed to delineating two neuronal systems of the CNS involved in regulating the BP. The first is related to the nucleus of the tractus solitarius (NTS) and mediates the depressor action of baroreceptors; the second is a distortion sensitive pressor system of the pontomedullary tegmentum and may be part of the so-called tonic vasomotor system of the lower brainstem. Of particular interest have been the examination of the effects of lesions of these systems on arterial pressure.

THE NTS SYSTEM

The NTS serves an important role in mediating some reflex actions on the circulation. Of critical importance is the intermediate one-third of the nucleus lying at the level of the obex. Electrophysiological and lesion data has supported the view that baroreceptor afferents from the carotid sinus and probably aortic and vagal nerves terminate there (e.g. Miura & Reis, 1970, 1972). It might be anticipated that lesions localized to the region of NTS would, like peripheral baroreceptor denervation, result in an elevated BP. Indeed in anaesthetized cat such lesions will entirely abolish the cardiovascular responses to receptor activation systemically as well as chemoreceptor responses to stimulation of carotid bodies (Miura & Reis, 1972). However, BP is not elevated.

Recently we sought to determine in *unanaesthetized* animals if NTS lesions will alter the BP. Initially such studies were done in rat (Doba & Reis, 1973; Doba & Reis, 1974; Reis, Doba & Amer, 1973). Bilateral lesions were placed in NTS in rats in whom appropriate cannulas for recording of BP and other cardiovascular responses were inserted and baseline values obtained in the unanaesthetized state. The animals were reanaesthetized with halothane, lesions placed in NTS, and anaesthesia discontinued. Within 15 min after termination of anesthesia the BP was elevated with systolic pressures over 200 mmHg. The elevated BP was not associated with changes in heart rate and entirely attributable to increased peripheral vasoconstriction consequent to augmented discharge of sympathetic vasomotor neurones (Doba & Reis, 1974). As a consequence of the augmented vasoconstriction, which closely engages vessels in skin, muscle, and all viscera except heart (Snyder, Doba, & Reis, unpublished), the cardiac output was reduced to almost 50% of control. Left ventricular pressure and central venous pressure were also elevated. Over hours, the BP and cardiac output gradually diminished until the animals developed cardiovascular collapse, congestive heart failure and died with pulmonary edema.

The central mechanisms which result in the elevated BP in rat after NTS lesions are not entirely known. As yet undefined structures lying above the caudal midbrain are necessary for its expression since the hypertension is abolished or prevented by midcollicular decerebration (Doba & Reis, 1973). Central catecholamine neurones are also involved in the

expression of hypertension but in a complex way. The development of hypertension can be blocked by intracisternal treatment with the adrenolytic agent 6-hydroxydopamine (6OHDA) probably through damage to bulbospinal tracts thereby indicating some adrenergic systems facilitate the BP. However, local injection of 6OHDA into NTS resulted in a moderate elevation of BP (Doba & Reis, 1974) suggesting other catecholamine terminals, probably in NTS, normally also depress BP.

Recently we have extended studies of NTS lesions to unanaesthetized cats hoping, because of a greater size and myocardial reserve, that they would survive. In these studies (Nathan & Reis, 1974) cannulas were implanted chronically in carotid artery and jugular vein and EEG, EMG and ocular electrodes implanted to monitor stages of sleep. After recovery, spontaneous and evoked fluctuations in BP and heart rate (HR) were observed over 1–2 weeks in these cats during various natural and elicited behaviors. Then, in a second operation, lesions were placed in NTS bilaterally. After NTS lesions cat, like rat, exhibited a marked elevation of BP within minutes after termination of anaesthesia. Unlike rats, however, cats survive the first 24 h and, after several days, mean BP drifted back to control levels becoming normal by 1 week after surgery. Such animals never exhibited a reflex bradycardia in response to baroreceptor activation indicating that the baroreceptor reflexes were destroyed permanently by the central lesion.

Although mean levels of BP were unchanged in NTS-lesioned animals, by 24 h after surgery, all NTS-lesioned cats exhibited a marked lability of arterial pressure which persisted for the longest period of observation (8 weeks). In lesioned animals the small *elevation* of BP observed during feeding, grooming, assumption of an upright posture, in response to petting, threat or the *fall* in BP occurring during the rapid eye movement (REM) phase of sleep were grossly exaggerated with shifts of 60–100 mmHg commonly occurring. Even at rest the BP, but not HR fluctuated widely suggesting that in these animals the BP is primarily regulated by changes in peripheral resistance and not cardiac output.

These results further support the view that NTS is an important relay station mediating the buffering action of baroreceptors on sympathetic neurones. Interference with the central neuronal mechanisms of this system moreover lead to labile hypertension in cat.

A PONTOMEDULLARY DISTORTION-SENSITIVE PRESSOR SYSTEM

We have in recent years been interested in several brainstem systems which mediate pressor responses. The identity of this system was recognized in a study by Hoff & Reis (1970) aimed at identifying the neuronal systems mediating the pressor response to elevated intracranial pressure, the so-called Cushing reflex. Hoff & Reis (1970) found that the area in the brainstem mediating the response was localized to a paramedian strip lying along the floor of the IVth ventricle. This was the only region of the brain from which the pattern of an elevated BP and reduction in HR were elicited by punctate distortion with a 1 mm probe.

Extending this study Doba & Reis (1972) demonstrated that, in cat, the Cushing response consisted of an elevated BP due to vasoconstriction in femoral, mesenteric and renal but not carotid arteries without change in cardiac output. A comparable response was elicited by either increasing the intracranial pressure diffusely by inflating a subdural balloon, by punctate distortion paramedially above the floor of the IVth ventricle, by the introduction

of small distorting pressures (< 10 cm H_2O) within a restricted area of dorsal tegmentum underlying the probe-sensitive area by the pulsed microinjection of 1–3 μ l of artificial CSF or by electrical stimulation within this area. The response area could therefore be mapped and was traced as a strip of neuronal tissue lying within the more widely representative ponto-medullary tegmental pressor areas (Wang & Ranson, 1939; Chai & Wang, 1962; Coote, Hilton & Zbrozyna, 1973). It is probable that in this area neurones sensitive to distortion (and possibly pressure and hypoxia) reside. By selective lesions we have found that the bradycardia is secondary to baroreceptor activation by the pressor response indicating that this system does not inhibit baroreceptors (Doba & Reis, unpublished). Stimulation of the system also elicits the release of adrenomedullary catecholamines. The region shares a similar localization with reference to the floor of the IVth ventricle as that area from which Claude Bernard elicited hypoglycaemia by puncturing the floor of the IVth ventricle in rabbits, a phenomenon known to be secondary to release of adrenaline.

The pathways mediating the Cushing response elicited by electrical stimulation are primarily uncrossed. Small lesions placed rostral, medial, or lateral to an electrode in the rostral pontine portion of the 'Cushing area' will not impair the magnitude of the response when elicited electrically; nor will it effect the mean BP. However, small lesions placed caudal to the lesion and only in the downstream portion of the Cushing area ipsilateral to the electrode entirely blocks the response and produces a fall of mean BP. A comparable lesion placed contralaterally while not altering the magnitude of the evoked Cushing response will also result in a fall of mean BP. Bilateral small caudal lesions will elicit a drop of mean BP to levels comparable to that found after transection of the spinal cord at the C1 level. These observations suggest that the Cushing area may represent the so-called tonically active vasomotor centres of the lower brainstem whose integrity is required for maintenance of normal levels of BP (Alexander, 1946; Reis, 1972).

The nature of the receptors mediating the Cushing response are unknown. The absence of morphologically identifiable receptors in the region suggest that it is the neurones themselves which are the distortion sensitive elements responding by excitation. The exquisite sensitivity of these neurones to distortion raises the possibility that small movements of brain which occur in life in response to arterial pulsations, respiratory movements, or simply movements of head may help to produce sufficient distortion and hence to produce background drive to the neurones in helping them maintain their tonic effects on BP. The possibility that heightened sensitivity of this system may also result in hypertension remains to be examined. However, in man and experimental animals there is evidence that chronic distortion of the floor of the IVth ventricle may lead to a sustained arterial hypertension (Reis & Doba, 1974).

ACKNOWLEDGMENTS

This work was supported by grants from NIH NS 03346, and NASA NGR 33-010-179.

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Fulminating Arterial Hypertension with Pulmonary Edema from Release of Adrenomedullary Catecholamines after Lesions of the Anterior Hypothalamus in the Rat

By Marc A. Nathan and Donald J. Reis

ABSTRACT

Bilateral electrolytic lesions of the anterior hypothalamus in unrestrained rats resulted in the development, within 2 hours, of arterial hypertension, tachycardia, hyperthermia, and increased locomotor activity, often leading to pulmonary edema and death. Similar lesions in paralyzed, artificially ventilated rats produced comparable changes in arterial blood pressure and body temperature with a similar time course. The arterial hypertension was a consequence of an increase in total peripheral resistance to 15% of control with a reduction in cardiac output to 49% of control. Arterial hypertension, elevated peripheral resistance, and diminished cardiac output were reversed toward normal by alpha-receptor blockade with phentolamine (1 mg/kg, iv). Bilateral adrenalectomy, adrenal demedullation, or adrenal denervation performed prior to lesion placement prevented the development of arterial hypertension and pulmonary edema as well as the changes in peripheral resistance, cardiac output, and body temperature. We conclude that arterial hypertension following lesions of the anterior hypothalamus is due to a neurally mediated increase in peripheral resistance initiated by the release of adrenal medullary catecholamines and that pulmonary edema is due to myocardial failure secondary to the ensuing ventricular overload. Structures originating in or passing through the anterior hypothalamus may exert selective control over the adrenal medulla independent of vasomotor neurons.

■ In 1954, Maire and Patton (1) demonstrated that bilateral electrolytic lesions of the anterior hypothalamus (AH) result in the development of a syndrome of hyperactivity, hyperthermia, and fatal pulmonary edema in the rat. Although cardiovascular measurements were not made, Maire and Patton attributed the pulmonary edema to a neurogenically mediated shift of blood from the capacitance vessels into the pulmonary circulation (2). More recently, Doba and Reis (3, 4) have observed that fulminating pulmonary edema can also be produced in the rat by bilateral lesions of the nucleus tractus solitarius (NTS), a brainstem nucleus in the medulla oblongata serving as a site of termination of arterial baroreceptors. In this instance, the pulmonary edema is due to the rapid development of neurogenic arterial hypertension with a marked increase in total peripheral resistance leading to a reduction in cardiac output and myocardial failure. Thus, the possibility exists that the pulmonary edema observed by Maire and

Patton (1, 2) after lesion of the AH was secondary to the development of arterial hypertension.

In the present study, we investigated the hemodynamic changes following placement of lesions in the AH in the rat. Such lesions produced pulmonary edema as a consequence of arterial hypertension, and the hypertension was due to a neurally mediated release of adrenomedullary catecholamines.

Methods

ANIMALS

Experiments were performed on Sprague-Dawley rats (Carr-Saunders Farms) of both sexes weighing 240–350 g. The rats were housed in groups of six in a light-cycled (on at 0700, off at 1900 hours), thermally regulated (20°C) room with free access to food and water.

STUDIES IN FREELY MOVING RATS

Measurement of Arterial Blood Pressure, Heart Rate, and Motility.—In the initial operation, rats were anesthetized with halothane (2–3% in a mixture of 50% O₂–50% N₂ blown over the nose through a face mask). For recording arterial blood pressure, one end of a polyethylene cannula (PE 50, 0.023 inches, i.d.) was inserted through the right common carotid artery into the ascending or the thoracic aorta. The other end of the cannula was brought out through the skin at a point midway between the scapulas and fed through a flexible metal spring attached to a saddle device strapped on the rat's back. The anesthesia was then discontinued, and the rat was placed in a plastic cage (47 × 20 × 25 cm). The free end of the spring cannula was attached to one end of a

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This work was supported by Grant NS 03346 from NINDS and Grant NGR-33-010-179 from NASA and by the Harris Foundation.

Received September 11, 1974. Accepted for publication May 13, 1975.

hydraulic swivel (BRS/LVE, Beltsville, Maryland). The other end of the swivel was connected by a tube to a strain-gauge transducer (Statham P23Db) connected in turn to a polygraph (Beckman type RM) for the display of arterial blood pressure. Heart rate was computed from the blood pressure pulse wave by a cardiometer (Beckman 9857) and simultaneously displayed. Motility was measured on an electromechanical counter as the number of times the rat interrupted a photoelectric beam at a point perpendicular to the long axis of the cage.

Placement of Anterior Hypothalamic Lesions.—After recordings of arterial blood pressure, heart rate, and motility had been taken for 1 hour, the rats were reanesthetized with halothane and placed in a stereotaxic frame. The dorsal surface of the skull was exposed, and the level of the head was adjusted by raising or lowering the incisor bar so that the tip of the electrode, positioned at anterior 7.8 mm relative to the interaural line, rested on the posterior lip of the bregma. After the skull had been opened, the electrode was moved 0.5 mm to the right or the left of the midline and then lowered to a point 1.4 mm above the interaural plane. Lesions were made by passing anodal constant current of 2–5 ma for 15–50 seconds through a monopolar stainless steel electrode (diameter 0.008 inches), which was insulated with Teflon to within 0.4 mm of the tip and supported in a 28-gauge stainless steel tube. The cathode was a clip attached to the adjacent temporal muscle. After placement of one lesion, the electrode was withdrawn, and a second lesion was placed at a homologous point in the brain on the other side of the midline. Anesthesia was discontinued, and the rats were removed from the stereotaxic frame and returned to the test cage. The cannula was then connected for an extended period of observation.

Several types of operated control rats were also prepared. In some rats, the brain was exposed but no lesions were made. In others, bilateral lesions were placed in the thalamus lying dorsal to the AH or in the cerebral cortex at the same rostral-caudal level as the AH.

Adrenal Surgery.—In selected rats, we assessed the possible contribution of adrenomedullary catecholamine secretion to the cardiovascular changes noted after AH lesions. Adrenomedullary release was impaired by bilateral adrenalectomy, adrenal demedullation, or adrenal denervation. After completion of the adrenal surgery, bilateral lesions were placed in the AH.

Adrenalectomy was performed through a flank incision after ligation of the vascular supply. Demedullation was performed by incising the adrenal cortex and then extruding the medulla by gentle pressure on the gland with smooth-tip forceps. The glands were denervated under a dissecting microscope through a flank incision by careful isolation of the gland under low-power magnification and separation of the fascicles coursing between the greater splanchnic nerve and the adrenal gland. When we suspected that the vascular supply to the gland had been compromised during the procedure, the rat was discarded. In control rats, the fascicles were isolated but left intact. Verification of the completeness of denervation was confirmed at autopsy.

STUDIES IN ARTIFICIALLY VENTILATED RATS

Measurement of Cardiovascular Dynamics and Method of Artificial Ventilation.—While the rats were *Circulation Research*, Vol. 37, August 1975

anesthetized with halothane, a stainless steel tracheal cannula was inserted below the larynx. A polyethylene cannula (PE 50) was inserted into the thoracic aorta via the left femoral artery and connected to a strain-gauge transducer (Statham P23Db) for the recording of blood pressure and heart rate on the polygraph. Another polyethylene cannula (PE 50) of known volume capacity was threaded up the left femoral vein into the right atrium to facilitate both the recording of venous blood pressure when the catheter was connected to another strain-gauge transducer (Statham P23Gb) or the introduction of saline for measurement of cardiac output (3). In rats in which cardiac output was measured, a thermistor (Hewlett-Packard model 15012) was inserted into the right common carotid artery and positioned in the aortic arch distal to the aortic valve. The locations of the tips of the thermistor and the venous cannula were always determined at autopsy. The rats were then placed in the stereotaxic frame, and bilateral electrolytic lesions were made in the AH as described earlier. In selected rats, a bilateral adrenalectomy was performed prior to the placement of AH lesions.

The rats were then paralyzed with curare (tubocurarine chloride, 0.4–0.8 mg/kg, iv). The tracheal tube was connected to a small-animal respirator (Harvard Apparatus Company no. 680), and the halothane was discontinued. To avert the hypoxia produced by the pulmonary atelectasis invariably associated with artificial ventilation of a rat with room air (5), these rats were artificially ventilated with a gas mixture of 50% O₂–50% N₂ at an average tidal volume of 1.75 ml and a respiratory frequency of 80/min (average minute volume of 140 ml). Tidal volumes were selected according to body weight by use of the nomogram of Kleinman and Radford (Harvard Apparatus Company).

Cardiac output was measured 2 hours after placement of the lesions by a thermal dilution technique (6–8) adapted for the rat and described in detail elsewhere (3). In brief, normal saline (0.1 ml) at room temperature was injected as a bolus into the right atrium through the venous cannula, and the temperature change of the bolus when it reached the aortic arch was measured with the thermistor. The resultant thermal dilution curve was displayed on the polygraph.

Blood Gas Analysis.—To ascertain that blood gases were within the physiological range in the paralyzed, ventilated rats, an 0.2-ml blood sample was withdrawn from the arterial cannula into a 1-ml heparinized glass syringe 2.5–3 hours after placement of the lesion. The oxygen tension (P_{O₂}), the carbon dioxide tension (P_{CO₂}), and the pH were measured in a Radiometer blood microsystem (type BMS3) (9).

OTHER PROCEDURES

Core body temperature before and after lesion placement was measured in some rats with a rectal thermistor probe connected to a temperature display unit (Yellow Springs model 8420). In other unlesioned rats, the core temperature was measured while they were exposed to an infrared heat source. In some experiments, phentolamine (1 mg/kg, iv) was administered in a volume that never exceeded 0.2 ml.

Great care was taken to ensure the comfort of the paralyzed unanesthetized rats. All wounds were packed with cotton saturated with aqueous procaine (2%), and

the eyes were covered with procaine ointment (5%). The inspired air was humidified. Body temperature was maintained at $38 \pm 0.5^\circ\text{C}$ through the use of a rectal probe connected to a thermostatically regulated electric heating pad. To ensure paralysis, needle electrodes were inserted into muscles of the posterior thigh, and the electromyogram was displayed on the polygraph; additional curare (0.4 mg/kg, iv) was administered as required. Control rats were prepared and maintained in a similar manner except that no lesions were made.

POSTMORTEM EXAMINATION

An autopsy was performed on all of the rats that died spontaneously or were killed by an intravenous or intra-arterial injection of sodium pentobarbital. After ligating the inferior and superior caval veins, the ascending aorta, and the trachea, the lungs were rapidly removed from the body and weighed. The lung weight/body weight ratio ($\times 100$) was used to assess the presence of pulmonary edema (3, 10).

The brain was removed and, along with the lungs, placed in 10% Formalin for at least 2 weeks. The localization of brain lesions was confirmed on frozen sections cut every 50μ and stained for cells by the Nissl method (11). The lungs were embedded in paraffin, stained with hematoxylin and eosin, and examined for pulmonary edema.

STATISTICAL EVALUATION OF DATA

The significance of changes in the cardiovascular responses and other variables resulting from brain lesions was determined by an unpaired *t*-test (12) or the Mann-Whitney *U*-test for independent samples (13). Changes were considered to be significant at $P < 0.05$.

Results

EFFECTS OF LESIONS OF THE ANTERIOR HYPOTHALAMUS IN FREELY MOVING RATS

The Syndrome.—Bilateral lesions of the AH in the rat invariably resulted in the gradual development of arterial hypertension, tachycardia, and increased motor activity (Fig. 1). The onset of hypertension (Fig. 1B) was evident 30 minutes after cessation of anesthesia, whereas heart rate (Fig. 1C) and motility (Fig. 1A) began to increase above control levels after 60 minutes. Arterial blood pressure and motility continued to increase over the next several hours; maximum elevations of blood pressure were achieved between 1 and 2.5 hours and of motility between 2 and 3 hours after placement of the lesion. In contrast, the tachycardia developed more gradually (Fig. 1C), reaching a maximum after 4 hours at a time when the arterial blood pressure was already declining (Fig. 1B).

At the time when arterial blood pressure and hypermotility were maximum, all of the rats displayed signs of intense autonomic arousal including piloerection, increased sweating, exophthalmia, and hyperthermia (Table 1). The rats were aggressive and irritable, and they would

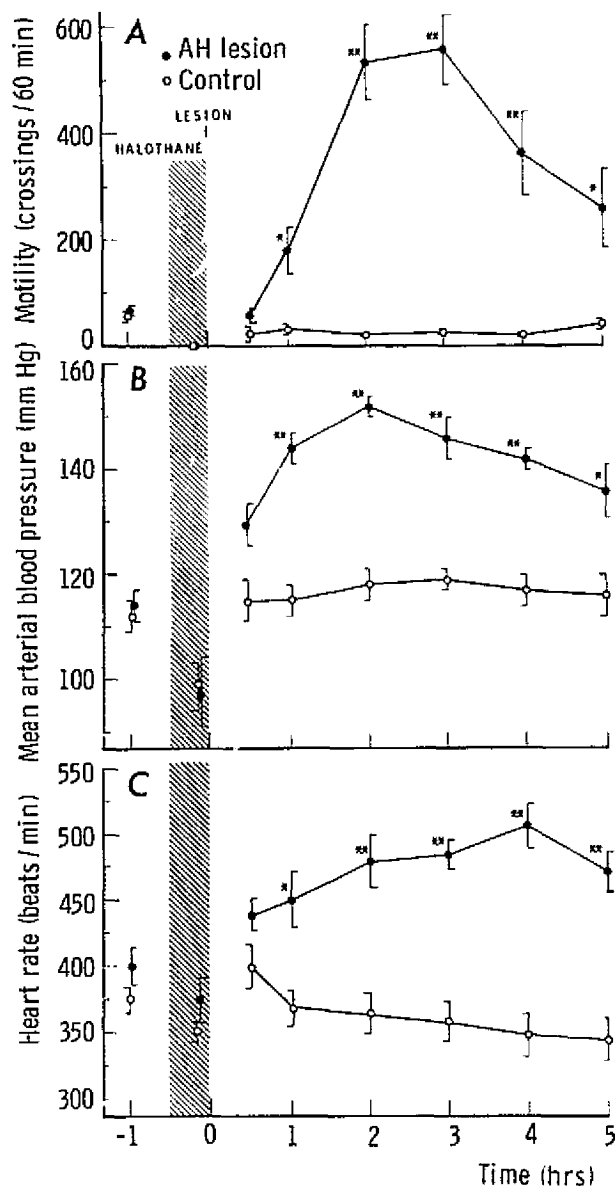


FIGURE 1

Changes over time in motility (A), mean arterial blood pressure (B), and heart rate (C) after bilateral electrolytic lesions of the anterior hypothalamus (AH) in the rat. Motility, arterial blood pressure, and heart rate were measured while the rats were unrestrained and freely moving. The rats were then anesthetized (cross-hatched bar), and at time 0 bilateral lesions were placed in the AH in the experimental group (solid circles, $N = 12$) or in the thalamus or the cortex in control group (open circles, $N = 13$). Each point signifies a mean value and bars indicate \pm SE. The significance of differences from control at each time point is represented by asterisks: * = $P < 0.01$, and ** = $P < 0.001$.

attack inanimate objects without hesitancy or provocation. When they were undisturbed, they ran compulsively without ceasing to rest or to consume food or water.

TABLE 1

Effects of Bilateral Lesions of the Nucleus of the Anterior Hypothalamus (AH) with and without Bilateral Adrenal Denervation or Adrenalectomy on Core Body Temperature

Experimental preparation	N	Temperature (°C)
Unparalyzed rats		
Control	7	37.9 ± 0.16
AH lesions	14	39.4 ± 0.34*
AH lesions + adrenalectomy	9	37.9 ± 1.01
Paralyzed rats		
Control	9	37.8 ± 0.22
AH lesions	7	39.0 ± 0.24*
AH lesions + adrenal denervation	5	38.5 ± 0.50

All values are means ± SE; N = number of rats tested. Rectal temperature was measured 2 hours after the lesions had been produced.

* $P < 0.01$ compared with the unparalyzed or the paralyzed control value.

The time required for the development of the arterial hypertension after placement of the lesions in the AH was delayed in comparison with the much more rapid evolution of hypertension in rats with NTS lesions (3, 4). We sought therefore to evaluate whether the delay was a consequence of unusually prolonged recovery from halothane. Bilateral lesions were placed in the AH in six rats, the anesthesia was discontinued, and arterial hypertension was permitted to develop. At 2 hours after placement of the lesions, when the circulatory

effects were maximum (Figs. 1B and 2A), the rats were reanesthetized for 20–30 minutes. As seen in Figure 2B, the arterial blood pressure of the AH-lesioned rats returned to control levels during anesthesia. However, 1–2 minutes after the anesthesia was terminated, arterial blood pressure began to rise. Within 7–10 minutes, the hypertension was reestablished. This experiment indicates that (1) arterial hypertension produced by lesions of the AH is reversible and sensitive to halothane anesthesia and (2) any lingering depressant effect of halothane on the cardiovascular responses to AH lesions is brief, lasting only 7–10 minutes. Therefore, the delayed development of hypertension after AH lesions cannot be attributed to halothane.

Arterial blood pressure and motility gradually began to fall (Fig. 1A and B) 3–4 hours after the placement of the lesions. Within 4–5 hours, 59% of the rats were dead and many others lay exhausted on the floor of the cage. Even though a number of the rats made futile attempts to run, they were unable to move themselves through the light beam, and the recorded motility decreased markedly (Fig. 1A).

The sequence of preterminal events in one of the rats that died is illustrated in Figure 3. Nearly 5 hours after the lesions had been placed and following a period of arterial hypertension, the arterial blood pressure fell precipitously to levels of 30–55 mm Hg, cardiac rate decreased, and cardiac ar-

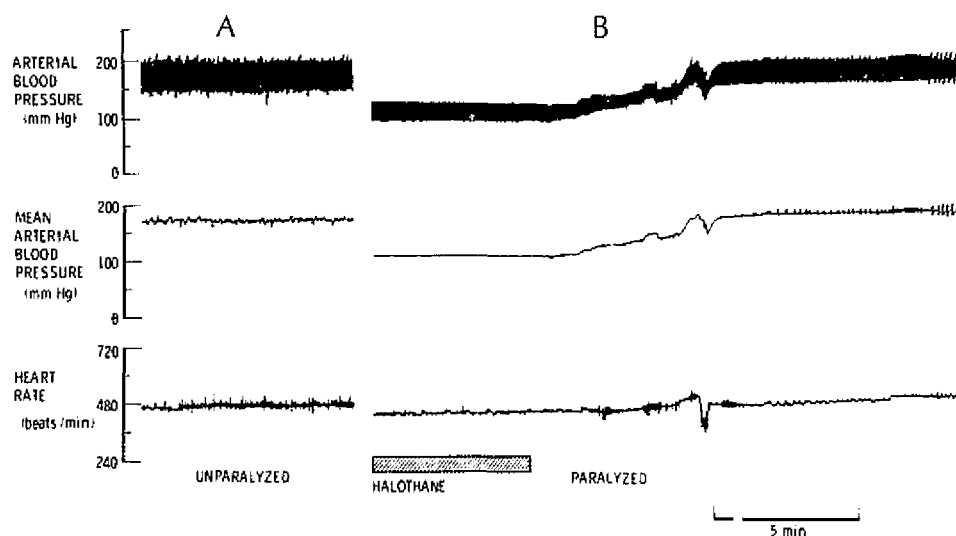


FIGURE 2

Effect of halothane anesthesia on arterial blood pressure and heart rate after AH lesions in the rat. Two hours after placement of AH lesions and discontinuation of halothane anesthesia, the hypertension was well established (A). The rat was reanesthetized, paralyzed, and artificially ventilated for 10 minutes, and the anesthetic was then discontinued (B). Note the rapid reestablishment of hypertension.

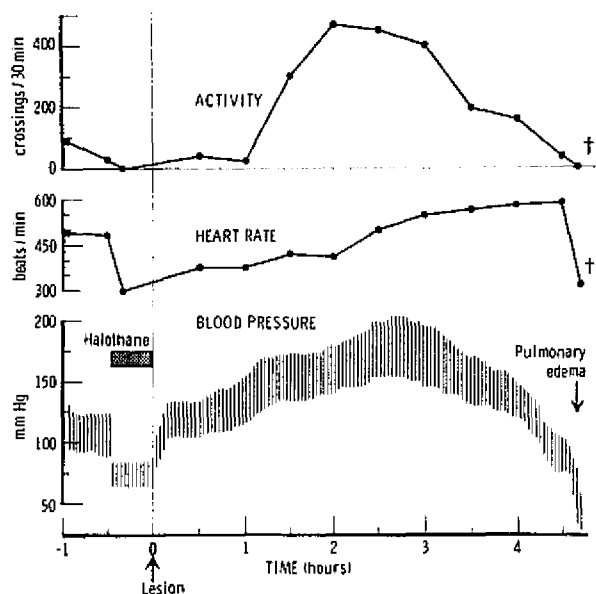


FIGURE 3

Changes in arterial blood pressure, heart rate, and motor activity in the unanesthetized, unrestrained rat before, during (cross-hatched bar), and following placement of bilateral electrolytic lesions of the AH. Just prior to death (indicated by the crosses), the rat developed pulmonary edema. The blood pressure response is schematized by the vertical lines: the height of each line represents the pulse pressure, and the top and the bottom of each line indicate the systolic and the diastolic pressure, respectively.

rhythmias developed; the rat lay motionless on the floor of the cage. At this time, clinical signs of pulmonary edema including labored breathing, audible gurgling, and the appearance of pink frothy fluid exuding from the nostrils were evident. At postmortem examination, this rat, as well as many of the other rats that died, had boggy lungs with frothy fluid often filling the trachea and the bronchi. The average lung weight-body weight ratio in the rats that died acutely 4-5 hours after placement of the AH lesions was significantly elevated (0.92 ± 0.06 , $N = 8$) compared with the control value (0.73 ± 0.4 , $N = 6$, $P < 0.05$). Microscopically, the lungs were characterized by an intense, diffuse interstitial and perivascular edema. Generalized vascular congestion and intra-alveolar edema were also often seen. These changes are characteristic of pulmonary edema (14) and identical to those occurring in rats following NTS lesions (3).

Localization of Effective Lesions.—In all instances, the electrolytic lesions effective in producing the hypertensive syndrome destroyed the bulk of the nucleus of the AH and the adjacent periven-

tricular nucleus of the hypothalamus. A typical lesion is illustrated in Figure 4. Rarely and variably, the lesions also damaged the adjacent optic tract, the fornix, the superchiasmatic nuclei, the caudal pole of the preoptic nucleus, or the rostral pole of the ventromedial hypothalamic nucleus. In two rats, the lesions were inadvertently placed asymmetrically in the AH. In these cases, one electrode penetrated the third ventricle and the other lodged in the lateral hypothalamus. The result was bilateral destruction of the periventricular nuclei and the medial part of the nucleus of the AH by the medial electrode and unilateral destruction of the lateral hypothalamic area by the lateral electrode. The degree of hypertension and motility developed by these rats was indistinguishable from that produced by the bilateral AH lesions. These observations suggest that destruction of either the medial portion of the AH or the periventricular nuclei may be responsible for the syndrome.

In the control rats, large lesions in the overlying thalamus, including the anterior medial nucleus, the reticular nucleus, and the ventral nucleus, did not result in changes in arterial blood pressure or motility. Lesions of the cerebral cortex were similarly without effect.

Effects of Adrenalectomy, Adrenal Demedullation, or Adrenal Denervation.—We next sought to examine the contribution of adrenal medullary catecholamine release to the syndrome produced by AH lesions. In the first of these particular experiments, we discovered that bilateral adrenalectomy performed just prior to placement of the AH lesions completely blocked the development of arterial hypertension but not the associated tachycardia and hypermotility produced by the AH lesions. Comparable effects were observed with selective adrenal demedullation or with bilateral denervation of the adrenal glands (Fig. 5). Preventing the release of adrenal catecholamines by any of these treatments attenuated the associated piloerection, proptosis, and mydriasis. Adrenalectomy also abolished the hyperthermia elicited by the lesions (Table 1). The findings therefore suggest that the arterial hypertension and some of the peripheral signs of sympathetic activation resulting from AH lesions are a consequence of the release of catecholamines from the adrenal medulla.

No evidence of pulmonary edema was seen in any of the rats in which adrenomedullary function was impaired, and lung weight-body weight ratios were normal (0.62 ± 0.02). However, 53% of these rats were dead 4-5 hours after lesion placement.

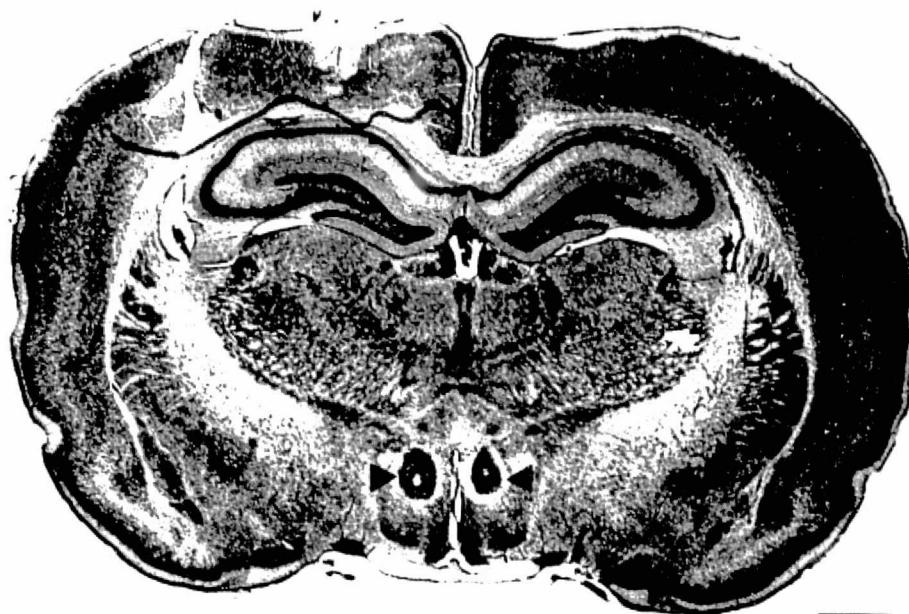


FIGURE 4

Location of lesions producing the AH syndrome in a representative coronal brain section at the level of the AH. Lesions are confined to the nucleus of the anterior hypothalamus (arrows) with some sparing of the ventral zone of the nucleus. Almost all of the adjacent periventricular nuclei was destroyed. The lesions extended rostrally to the preoptic area and caudally to the nuclei of the ventromedial hypothalamus. Cresyl violet stain. Bar = 1 mm.

The high rate of mortality may have been due to the stress of running, since all of the paralyzed, adrenalectomized rats with AH lesions survived.

Effects of Hyperthermia.—To assess the effects of elevation of body temperature on arterial blood

pressure, four unlesioned rats were exposed to an infrared heat source until their body temperatures were elevated to $40.4 \pm 0.21^\circ\text{C}$. The mean arterial blood pressure recorded from these rats at this time was only 114 ± 3 mm Hg. Thus, it is unlikely that the changes in arterial blood pressure observed after AH lesions were a consequence of the accompanying hyperthermia.

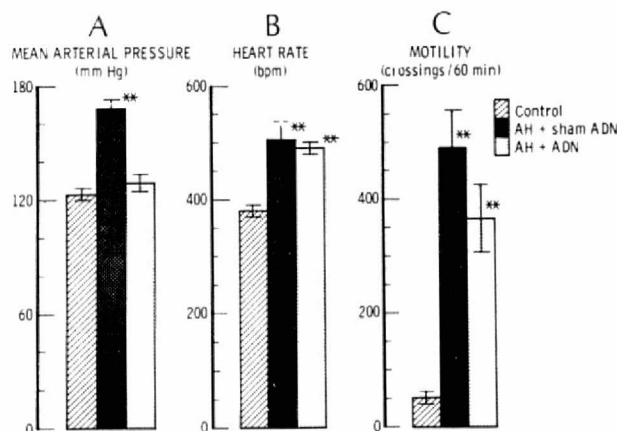


FIGURE 5

Effects of total bilateral denervation of the adrenal glands (ADN) on mean arterial blood pressure (A), heart rate (B), and motility (C) produced by lesions of the anterior hypothalamus (AH). Note that adrenal denervation but not a sham-operation blocked the development of hypertension measured 2 hours after the lesion. Arterial blood pressure was measured as the mean \pm SE for six rats. ** = $P < 0.001$ compared with control.

EFFECTS OF LESIONS OF THE ANTERIOR HYPOTHALAMUS IN PARALYZED RATS

Changes in Arterial Blood Pressure, Heart Rate, and Body Temperature.—Next, we studied the effects of AH lesions in paralyzed, artificially ventilated rats to assess the contribution of muscular movement to the changes in arterial blood pressure, heart rate, and body temperature. As illustrated in Figure 6, bilateral lesions of the AH in paralyzed rats resulted, after discontinuation of the anesthetic, in the development of arterial hypertension comparable to that seen in the unrestrained rats (Fig. 1B) and significantly different from that seen in the paralyzed controls, who tended to develop a mild elevation of arterial blood pressure. Although the heart rate also increased in lesioned, paralyzed rats, the differences from controls were not significant, because the control rats gradually developed a moderate tachycardia. The

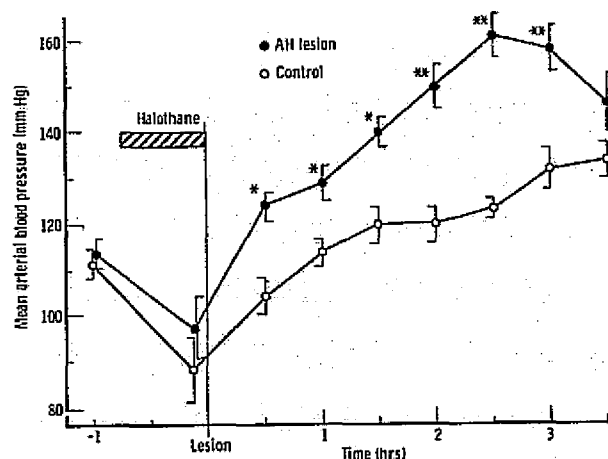


FIGURE 6

Changes in arterial blood pressure in paralyzed, artificially ventilated, lesioned rats (solid circles, $N = 13$) compared with those in sham-operated, paralyzed controls (open circles, $N = 12$). Note that the time course for the development of arterial hypertension after anterior hypothalamus (AH) lesions in paralyzed rats is identical to that in unrestrained rats (Fig. 1). * = $P < 0.01$ compared with control, and ** = $P < 0.001$ compared with control.

lesioned, paralyzed rats were also hyperthermic (Table 1); they exhibited a mean body temperature almost identical to that observed in the unrestrained, lesioned rats and significantly different from that seen in the paralyzed, unlesioned controls. These findings indicate, therefore, that the lesion-elicited hypermotility was not the cause per

se of the cardiovascular changes or the hyperthermia.

Cardiodynamic Changes during the Acute Hypertensive State.—We next sought to determine if the arterial hypertension produced by AH lesions was due to an increase in total peripheral resistance or to an increase in cardiac output. The cardiodynamic changes were measured in control rats and in rats with AH lesions 2 hours following cessation of the anesthetic at a time when the arterial hypertension was fully developed.

As indicated in Table 2, AH lesions in paralyzed rats resulted in significant elevations in systolic, diastolic, and mean arterial blood pressures with an increase in total peripheral resistance to 157% of control and a reduction in cardiac output to 49% of control. Central venous pressure was unchanged. The cardiovascular changes could not be attributed to alterations in blood gases, which were within normal limits.

Effect of Phentolamine on Arterial Blood Pressure, Cardiac Output, and Total Peripheral Resistance.—Administration of the alpha-adrenergic blocking agent, phentolamine (1 mg/kg, iv), immediately reversed toward normal the arterial hypertension, the elevation in total peripheral resistance, and the decrease in cardiac output resulting from bilateral lesions of the AH (Fig. 7). Heart rate remained unchanged. These findings indicate that the low cardiac output characterizing the acute hypertensive state associated with AH lesions is

TABLE 2

Effects of Bilateral Lesions of the Nucleus of the Anterior Hypothalamus (AH) with and without Bilateral Adrenalectomy on Cardiovascular Dynamics and Blood Gases

Variable	Control	AH lesions	AH lesions + adrenalectomy
Systolic arterial blood pressure (mm Hg)	161 \pm 5 (9)	183 \pm 7* (9)	144 \pm 13 (4)
Diastolic arterial blood pressure (mm Hg)	122 \pm 6 (9)	142 \pm 5† (9)	118 \pm 8 (4)
Mean arterial blood pressure (mm Hg)	133 \pm 4 (12)	159 \pm 4‡ (13)	130 \pm 6 (5)
Heart rate (beats/min)	399 \pm 21 (12)	440 \pm 21 (13)	529 \pm 22§ (5)
Cardiac output (ml/min)	90 \pm 21 (6)	44 \pm 4* (6)	65 \pm 8 (5)
Total peripheral resistance (mm Hg·min/ml)	2.000 \pm 0.396 (6)	3.138 \pm 0.671* (6)	2.092 \pm 0.156 (5)
Central venous pressure (cm H ₂ O)	2.4 \pm 0.80 (6)	2.5 \pm 0.36 (6)	1.4 \pm 0.52 (5)
Arterial Po ₂ (mm Hg)	96 \pm 9 (7)	104 \pm 6 (9)	158 \pm 15 (4)
Arterial Pco ₂ (mm Hg)	39 \pm 3 (7)	40 \pm 3 (9)	
Arterial pH	7.37 \pm 0.01 (7)	7.36 \pm 0.02 (9)	7.42 \pm 0.09 (3)

All cardiovascular measurements were made in paralyzed, artificially ventilated rats 2 hours after placement of lesions in the AH. Adrenalectomy was performed just prior to placement of the lesions. Blood gas measurements were made 2–3 hours after lesion production. Due to technical difficulties, Pco₂ measurements from rats with AH lesions + adrenalectomy could not be made. All values are means \pm SE; the number of rats in each group is given in parentheses.

* $P \leq 0.05$ compared with control.

† $P \leq 0.02$ compared with control.

‡ $P \leq 0.001$ compared with control.

§ $P \leq 0.01$ compared with control.

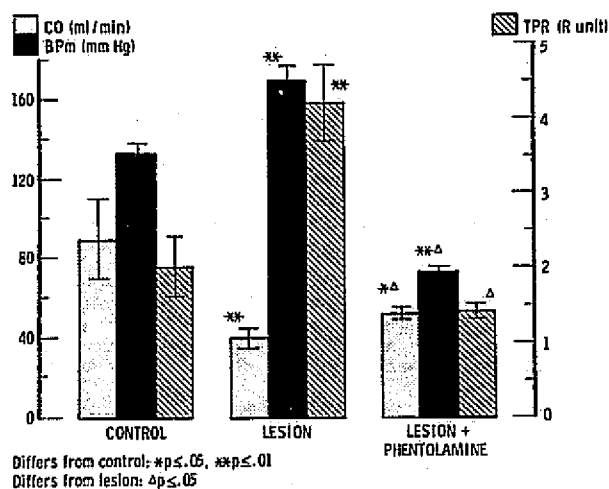


FIGURE 7

Effects of phentolamine (1 mg/kg, iv) on mean blood pressure (BPm), cardiac output (CO), and total peripheral resistance (TPR) in rats with hypertension produced by bilateral lesions of the AH. Rats were anesthetized with halothane and cannulated, and lesions were placed in the AH. The rats were then paralyzed and artificially ventilated. The control rats were similarly treated except that no lesions were made. Two hours after discontinuation of the anesthetic, measurements were obtained in all rats. Phentolamine was then administered to the rats with lesions, and cardiovascular activity was determined within the next 10 minutes. Note the reversal of hypertension, the decrease in total peripheral resistance, and the increase in cardiac output produced by the drug.

secondary to the increased peripheral vascular resistance, which in turn is mediated by stimulation of alpha receptors.

Effects of Acute Bilateral Adrenalectomy on Cardiovascular Changes Initiated By AH Lesions.—In paralyzed rats, bilateral adrenalectomy performed just prior to the placement of the brain lesion entirely blocked the elevation of arterial blood pressure and the increase in total peripheral resistance produced by the AH lesions (Table 2). The lesioned, adrenalectomized, paralyzed rats had elevated heart rates compared with those of the unlesioned, paralyzed controls (Table 2). Adrenalectomy also blocked the development of hyperthermia (Table 1), indicating thereby that the hyperthermia, like the hypertension, depends on the release of adrenal catecholamines.

Discussion

The present study demonstrates that bilateral electrolytic lesions of the AH in the rat result in the development of acute arterial hypertension, often leading, within hours, to pulmonary edema and death. Although the acute changes in arterial blood pressure and heart rate that follow lesions of the

AH have not been previously described, the other manifestations of the lesions, particularly pulmonary edema as well as increased locomotor activity, hyperthermia, widespread sympathetic activation, and emotional hyperreactivity, have been described by Maire and Patton (1, 2, 15) following the placement of lesions in regions variously identified as the preoptic area (2) or the rostral hypothalamus (15). Since these regions, according to current opinion, are part of the AH (16), the pulmonary edema produced by Maire and Patton and the edema observed in the present study were probably caused by placement of lesions in the same general area. It is also highly probable that the pulmonary edema reported by Maire and Patton was preceded by a period of arterial hypertension.

The arterial hypertension produced by AH lesions is, like that produced by NTS lesions, entirely due to increased peripheral resistance and not to an elevation of cardiac output. Indeed, cardiac output is reduced by half, probably as a consequence of a reduced stroke volume resulting from the ventricular overload imposed by the elevated peripheral resistance, since reduction of peripheral resistance and arterial blood pressure by alpha-receptor blockade returns the cardiac output toward normal levels. Pulmonary edema appeared as a terminal event in over half of our rats 4–5 hours after placement of the lesions. It was probably the consequence of left heart failure with subsequent congestion of the pulmonary circulation, perivascular edema, and, terminally, exudation of fluid into the alveolar spaces.

The arterial hypertension as well as some of the peripheral effects of sympathetic activity depend on a neurally mediated release of catecholamines from the adrenal glands and can be blocked by adrenal surgery. Moreover, the release of catecholamines appears to be a direct result of the AH lesion and not secondary to associated components of the syndrome. It is not a consequence of muscular activity, since hypertension was produced by AH lesions in paralyzed rats. It is not due to changes in blood gases, possibly consequent to catecholamine release (17), since these were normal at a time when the rats were hypertensive, and it is probably not due to the elevated body temperature, since heating rats to over 40°C was not associated with hypertension.

The absence of any elevation in arterial blood pressure after lesions are placed in rats with adrenal denervation suggests that the AH lesions initiate a differentiated activation of the sympathetic nervous system with a preponderant dis-

charge of preganglionic sympathetic neurons to the adrenal gland. This pattern is entirely different from that associated with the hypertension produced by NTS lesions. In the latter instance, the hypertension appears primarily to result from augmented sympathetic vasomotor activity with little adrenal involvement (3).

The central mechanisms through which AH lesions lead to the development of the AH syndrome are uncertain. One interpretation (15) is that structures either originating in or passing through the region of the AH exert a tonic inhibitory effect on other brain areas which themselves provide excitation for the neural networks mediating component parts of the syndrome. One such area which might be disinhibited by the AH lesion is the adjacent lateral hypothalamus. There are known anatomical connections between the lateral and the medial hypothalamus (18), and electrical stimulation of the antero- and the posterolateral hypothalamus has been demonstrated to produce a release of adrenomedullary catecholamines (18-21). However, if the AH syndrome is simply due to disinhibition, then it is difficult to explain the long latency required for the full development of the responses; we have clearly established that it is not attributable to anesthesia.

Another possibility is that the AH syndrome is due to lesion-induced excitation rather than disinhibition of pathways responsible for the expression of the observed behaviors. Excitation might result from the slowly accelerating release of neurotransmitters from the terminals of damaged axons, degeneration activation (22), or from the deposition of iron at the electrode site, which has been implicated in producing prolonged excitatory effects in the central nervous system (23-26).

Whatever the mechanism responsible for the production of the AH syndrome, the fact remains that elimination of the adrenal medullary secretion can entirely abolish the arterial hypertension associated with the AH lesion. That fact is of considerable importance in the framework of the central neural organization of the cardiovascular system. The finding suggests, first, that the central neural representations of the adrenal medulla and the vasomotor systems are in part distinct. This feature of the central organization of the autonomic nervous system is not widely appreciated, but it is implicit in findings that adrenal catecholamines may be released reflexively through activation of receptors within the central nervous system by hypoglycemia induced by insulin or 2-deoxy-D-glucose (26-32) in the absence of significant hypertension (27). Second,

it indicates that pathways originating in or passing through the AH exert control over the adrenal medulla, thereby pointing to a specific area of the brain involved in adrenomedullary control.

Acknowledgment

We thank Dr. Pierre Gauthier for help in several of the experiments. Nancy Shieh provided excellent technical assistance.

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TWO MODELS OF ARTERIAL HYPERTENSION IN RATS PRODUCED BY LESIONS OF INHIBITORY AREAS OF BRAIN

DONALD J. REIS, MARC A. NATHAN,
NOBUTAKA DOBA, AND M. SAMIR AMER

Two models of acute fulminating arterial hypertension can be produced by rats by brain lesions which destroy central inhibitory pathways. The first, nucleus tractus solitarius (NTS)-hypertension, is a consequence of central deafferentation of baroreceptors made by lesions placed at the site of their termination in the brain in NTS. This mode of hypertension develops immediately following recovery from anesthesia and is characterized by increased total peripheral resistance and an associated fall of cardiac output. It is mediated entirely by increased activity of sympathetic vasomotor fibers. The central pathways mediating the hypertension are not known but depend upon the integrity of structures lying above the midbrain and also of descending noradrenergic tracts. Within two hours following the onset of increased sympathetic vasomotor activity, changes can be observed within the cyclic nucleotide metabolism of blood vessels and heart with a decrease of cyclic AMP and an increase of cyclic GMP occurring in the aorta, and a decrease of cyclic AMP occurring in the heart. The alterations of cyclic AMP are the result of increased degradation, while the elevated GMP levels result from increased synthesis. Such changes in cyclic nucleotides may be related to triggering mechanisms which lead to enduring changes in the metabolism of blood vessels which may serve to fix the hypertension.

This research was supported by Grants from the National Institute of Health, NS 04876, NS 3346, and from NASA, NGR 010-179.

The second syndrome of hypertension can be produced by lesions of the anteromedial hypothalamus (AH-hypertension). The hypertension in this instance develops after a latency of sixty to ninety minutes, is associated with markedly increased motor activity, hyperthermia, and generalized sympathetic arousal. It is abolished by bilateral adrenalectomy, by adrenal demedullation, and selective adrenal denervation. It therefore appears to be the consequence of interruption of fibers originating in or passing through the anterior hypothalamus which selectively inhibit the release of adrenomedullary catecholamines.

Our findings indicate that selective interruption of central systems modulating sympathetic activity can lead to arterial hypertension. Such models of acute hypertension in rats may be useful in further analyzing central neural mechanisms governing the discharge of the central nervous system, and which might be impaired in the human counterpart of the disease.

INTRODUCTION

THERE IS INCREASING EVIDENCE, as attested to by other papers in this Symposium, that the sympathetic nervous system may play a critical role in initiating or sustaining the disease of essential hypertension in man. The precise relationship between the augmented sympathetic nerve activity and arterial hypertension, however, remains to be elucidated. One view(1, 2) has proposed that increased states of sympathetic nerve activity, perhaps governed by heightened states of vigilance or emotionality can, when sustained, lead to structural or biochemical changes in blood vessels. These vascular changes, possibly leading to a reduction in the wall-lumen ratio(2), could result in a sustained increase of vascular resistance. Thus, reestablishment of normal levels of sympathetic discharge would *not* result in immediate reversal of the increased vascular resistance in arterial pressure. Such transmutation of a transient to an established state of heightened vascular resistance might serve as a link between neurogenic and essential forms of hypertension.

Indeed, prolonged arterial hypertension persisting long after the cessation of an initiating stimulus has been observed in several experimental models in which arterial hypertension has been produced by emotional or physical stress(3-5), by conditioning(6), or by repetitive electrical stimulation of the brain(7).

It is evident that any consideration of a link between the sympathetic nervous system and hypertension must consider the role

of the central nervous system as a site of disordered function that ultimately mediates sympathetic nervous effects on blood vessels. It has long been recognized that the CNS modulates the tonic level of sympathetic discharge as well as phasic changes in response to local metabolic requirements or in response to more general homeostatic drives(8, 9). By suitable integration of excitatory and inhibitory inputs modulating sympathetic nerve discharge arising from widely dispersed segments of the neuraxis, the CNS produces an orderly matching of cardiovascular events appropriate to specific behaviors. Potential disorders in this balance could set the conditions favoring the development of augmented sympathetic discharge, with increased vascular resistance and tone leading to arterial hypertension. The role of CNS in development of hypertension has recently been reviewed(10).

It is obvious then that the hypertension consequent to increased sympathetic neural discharge can result from conditions either resulting in direct excitation or withdrawal of inhibition from sympathetic neurons. In the past, attempts to produce hypertension by manipulations of the CNS have favored techniques whereby sympathetic neuronal activity has been enhanced by environmental stimuli, e.g. chronic stress or conditioning(3-6), or by electrical stimulation of the brain(7). Hypertension resulting from withdrawal of inhibition to sympathetic neurons has been principally produced by attempting to withdraw the inhibitory input from peripheral baroreceptors by sinoaortic denervation(1, 10). Such hypertension, which has been observed in rats, cats, dogs, and rabbits is usually moderate and not usually sustained.

Recently, we have attempted to develop several models of arterial hypertension in rats by lesioning regions of the brain which appear to exert a tonic inhibition of the sympathetic nervous system(11-14). These inhibitory regions include the region of nucleus tractus solitarii (NTS) lying at the obex, a primary site of termination of baroreceptor input(15-20), and also lesions of anteromedial hypothalamus, a region which has been identified as being a sympatho-inhibitory area(21-23). We have observed that bilateral small electrical lesions in either area will produce fulminating arterial hypertension in rat but by different mechanisms.

Moreover, we have discovered that hypertension produced by NTS lesions may result in striking changes in cyclic nucleotide metabolism(13) which may serve to trigger long-term metabolic and structural changes in blood vessel walls.

**NEUROGENIC HYPERTENSION RESULTING FROM
CENTRAL DEAFFERENTATION OF BARORECEPTORS:
NTS HYPERTENSION IN RAT**

The Syndrome

The recent discovery of the central projections of baroreceptor afferents to the nucleus tractus solitarii (NTS)(16-20) and the discovery by Miura and Reis(20) that small bilateral lesions at the level of the obex will abolish all baroreceptor reflexes led us(11) to examine whether or not neurogenic hypertension could be produced in the rat by central deafferentation of baroreceptors. Our studies were performed on rats in whom intravascular can-

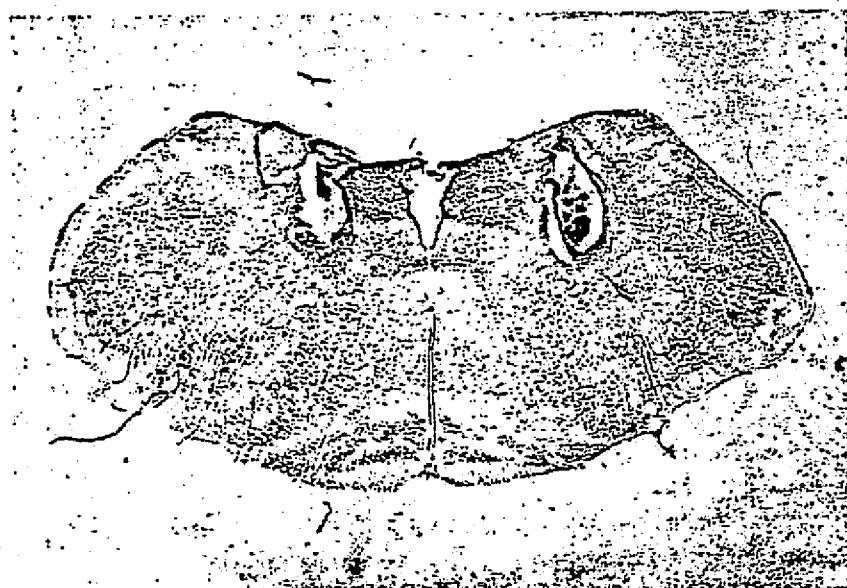
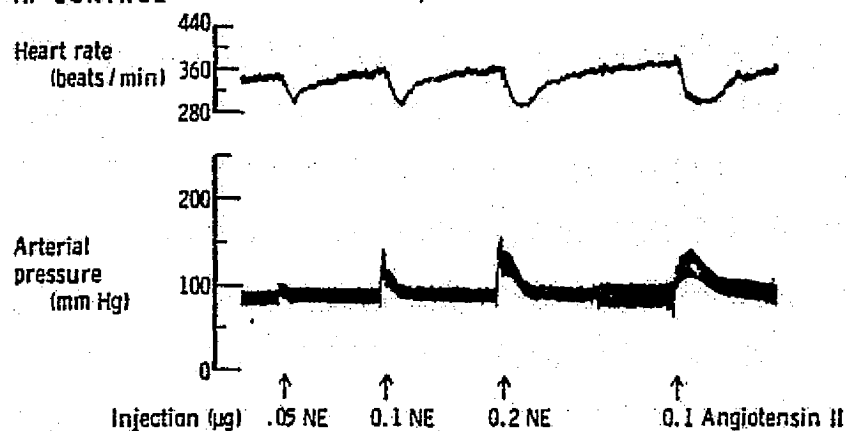
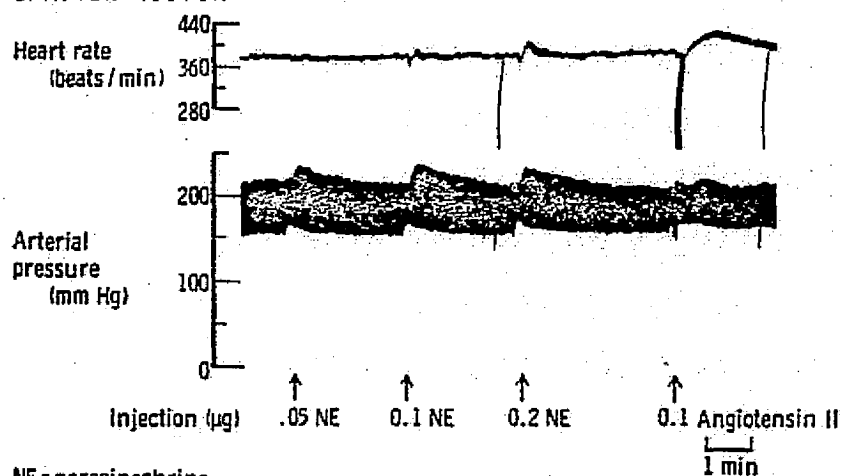


Figure 7-1. Representative lesion of the brainstem in a rat that produced neurogenic hypertension. This section was taken just rostral to the obex. Nissl stain. The lesion destroys the NTS bilaterally. (Courtesy of *Circulation Research*[11]).

A) CONTROL



B) AFTER LESION



NE = norepinephrine

Figure 7-2. Reflex bradycardia and systemic hypertension evoked by intravenous injection of different doses of norepinephrine (NE) and angiotensin II before (A) and after (B) production of bilateral lesions of the NTS in the anesthetized rat (α -chloralose 30 mg/kg IV). Before lesions, both agents produced a graded bradycardia associated with hypertension. After lesions, the reflex bradycardia was no longer elicited, and tachycardia and arrhythmias were observed with injections of norepinephrine and angiotensin II, demonstrating that baroreceptor reflexes were abolished. (Courtesy of *Circulation Research*[11]).

nulae were inserted while the animal was briefly anesthetized with halothane. Basal cardiovascular activity was determined following discontinuation of the anesthesia. The animal was reanesthetized with halothane and small bilateral lesions placed in the NTS. The animal was allowed to recover from anesthesia and cardiovascular function was followed.

We discovered that lesions destroying NTS bilaterally (Fig. 7-1) and which abolished all baroreceptors reflexes (Fig. 7-2) invariably resulted in arterial hypertension. The hypertension appeared within five minutes after halothane anesthesia was discontinued and by thirty minutes had achieved maximal levels (Fig. 7-3).

The arterial hypertension produced by NTS lesions can be entirely attributed to increased peripheral resistance. The elevated resistance produces an overload of the left ventricle, a consequent

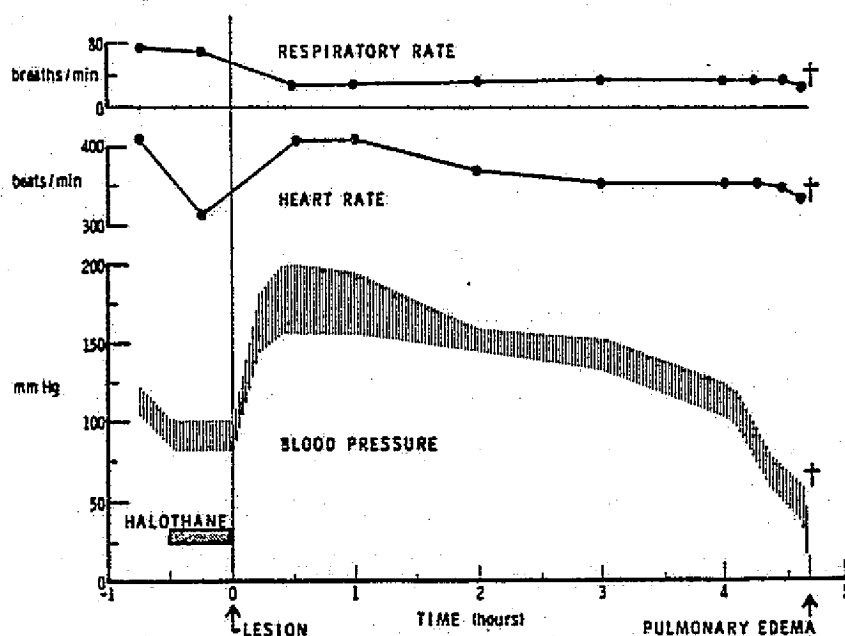


Figure 7-3. Time course of changes in systemic arterial pressure, heart rate, and respiratory rate in a representative unanesthetized rat after production of bilateral lesion of NTS. Just prior to death the animal developed pulmonary edema. (Courtesy of *Circulation Research*[11]).

reduction of stroke volume, and an increase in end-diastolic pressure resulting in a fall of cardiac output to around 60 percent of normal. Central venous pressure is also elevated. The elevated peripheral resistance and incipient congestive failure ultimately evolve and by four to six hours after placement of the lesions, the animals rapidly develop acute pulmonary edema and die.

The hypertension produced by NTS lesions is neurogenic, and is due to a differentiated activation of sympathetic neurons to blood vessels since there is no evidence of widespread sympathetic activation. It is not attributable to changes in blood gases nor to the release of pressor substances from the kidneys or the adrenal glands (even though some adrenal medullary catecholamines are released)(12), since prior adreno-nephrectomy does not reduce the magnitude of the hypertension(11). On the other hand, ganglionic blocking agents or the α -adrenergic blocking agent phentolamine will reduce the elevated blood pressure and reverse the cardiac failure(12).

Central Neural Pathways

All evidence suggests that NTS lesions produce their release of sympathetic neural activity by damaging the central projections of baroreceptors in NTS. The hypertension, however, is more intense than that produced by sinoaortic denervation alone probably because the central lesion interrupts additional depressor reflexes arriving from the cardiopulmonary mechanoreceptors which traverse the vagus nerve(25) and which are usually left intact when the sinoaortic nerves are cut. It also may interrupt intrinsic CNS pathways acting at the level of NTS and modulating the baroreceptor responses(10, 25-27). It is extremely unlikely to be the consequence of any irritative (i.e. stimulatory) effect of the lesion(11).

One of the most characteristic features of the hypertension evoked by NTS lesions is its dependence on the integrity of structures lying above the midbrain. Midcollicular decerebration will abort the development of hypertension before NTS lesions are placed or abolish the hypertension once the lesions are established (11). The importance of rostral regions of the brain in mediating the hypertension parallels the observations that the reflex hypertension elicited by sinoaortic denervation or carotid occlusion

is reduced or abolished by decerebration(28, 29). The findings support the contention that baroreceptors, after terminating in the medulla, engage in long-loop cardiovascular reflexes with higher brain areas(18). The precise localization of the rostrally situated regions necessary for this mode of hypertension remains to be established.

Central Catecholamines in Neurogenic Hypertension

While the precise pathways mediating NTS-hypertension remain to be elucidated, there is evidence(12) that the expression of NTS-hypertension depends on the integrity of at least one of the central neuronal systems which synthesize, store, and release the catecholamine neurotransmitters dopamine and norepinephrine. Intracisternal injection of 6-hydroxydopamine (6-OHDA), a drug which will destroy noradrenergic neurons, blocks the development of NTS-hypertension (Fig. 7-4) even when the adrenal glands are intact(12). This finding is consistent with the observations of Chalmers and Reid(30) indicating that intracisternal 6-OHDA aborts the hypertension produced by sinoaortic denervation in rabbits. It is not certain which central catecholamine systems are involved. Most likely, it is a noradrenergic rather than dopaminergic system since bilateral intrahypothalamic injection of 6-OHDA, which interrupts major dopaminergic as well as noradrenergic projections to hypothalamus and other forebrain structures (31, 32), fails to influence the NTS-hypertension. It is more likely that a bulbo-spinal system is critical for the expression of the hypertension since with low doses of 6-OHDA the only region showing depletion of amines is the spinal cord. Our conclusion is in agreement with that of Chalmers and Reid(30), indicating that bulbospinal noradrenergic systems support the neurogenic hypertension produced by sinoaortic denervation in rabbits.

On the other hand, not all cortical noradrenergic systems facilitate the development of hypertension. Local injection of 6-OHDA directly into the area of NTS will result only in a transient hypertension(12) (Fig. 7-5). This finding indicates that not all noradrenergic systems facilitate the arterial pressure. Indeed, it suggests that some systems may serve to depress it. The NTS and adjacent medial dorsal regions of the medulla in rat are richly innervated

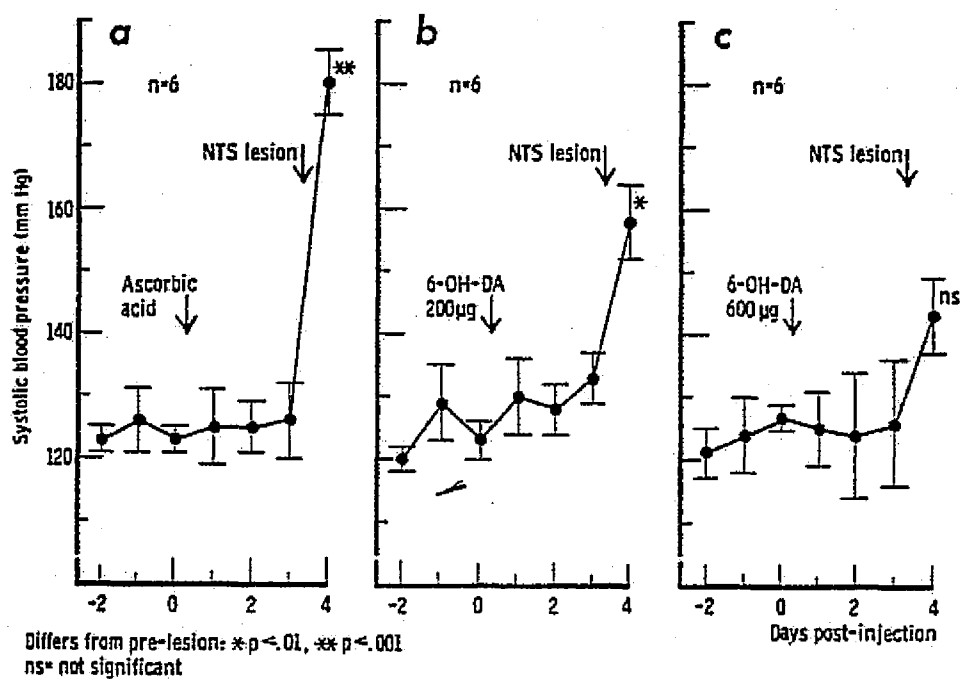
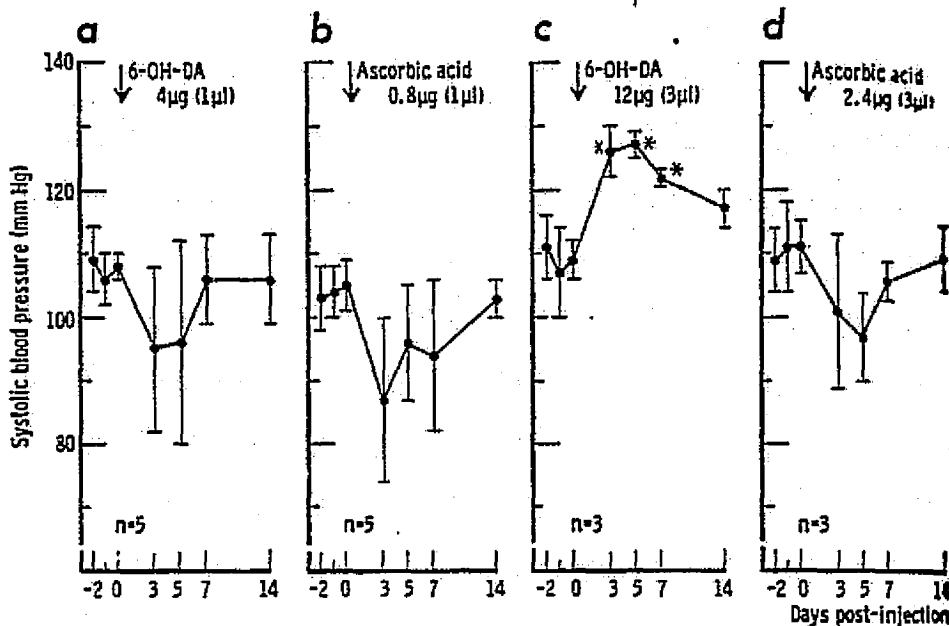


Figure 7-4. Effects of 6-OHDA administered intracisternally on systolic blood pressure before and after NTS lesions. Blood pressure was measured by a tail cuff method for three consecutive days before the intracisternal injection of 6-OHDA. Control rats (a) were treated with ascorbic acid vehicle alone. Other rats received 200 μ g (b) and 600 μ g (c) of 6-OHDA in 10 μ liters of ascorbic acid vehicle. Blood pressure was measured for four days and then bilateral lesions of the NTS were placed. Note that 6-OHDA in the higher dose blocked and in the lower dose attenuated the NTS-hypertension. (Courtesy of *Circulation Research*[12]).

with noradrenergic terminals and also contain some cell bodies of noradrenergic neurons(31, 33). The role of noradrenergic innervation of NTS is unknown. However, recent studies on the pharmacological action of the centrally acting hypotensive agent, clonidine, a drug believed to act as an α -adrenergic agonist, have suggested that norepinephrine may produce its hypotensive actions by activation of baroreceptor pathways possibly within the NTS (34, 35). Thus norepinephrine may have opposing central actions on the arterial pressure depending on the site at which it is re-



* Differs from control $p < 0.01$

Figure 7-5. Effects on systolic blood pressure of 6-OHDA directly microinjected into the area of the NTS. Blood pressure was measured by a tail cuff method. Blood pressure was observed for three consecutive days before and for fourteen days after the microinjections of the drug into the NTS areas. A small dose (a) of 6-OHDA (4 μ g in 1 μ liter) did not produce any significant changes in blood pressure. A larger dose (c) of 6-OHDA (12 μ g in 3 μ liters) produced a significant rise in blood pressure which gradually returned to control levels after fourteen days. Rats treated with either 1 μ liter (b) or 3 μ liters (d) of ascorbic acid vehicle alone did not show any change in blood pressure. (Courtesy of *Circulation Research*[12]).

leased, the origin of the parent cell body, and, possibly, the nature of the receptor, whether α or β . Our findings suggest that in NTS norepinephrine opposes while in the spinal cord it facilitates a rise of blood pressure.

It is, however, unlikely that the whole syndrome of NTS-hypertension, when produced by electrolytic lesions of NTS, can be explained *exclusively* on the basis of destruction of only noradrenergic terminals in NTS. First, in contrast to NTS-hyperten-

sion, the effect produced by microinjection of 6-OHDA into NTS is mild and transient. Second, NTS-hypertension is similar to that produced by selective denervation of baroreceptors, yet baroreceptor afferents are not noradrenergic. It is more probable that the noradrenergic innervation of the NTS primarily modulates baroreceptor reflex mechanisms rather than serving as the primary activator of the reflex.

Cyclic Nucleotide Metabolism in Blood Vessels and Heart in Neurogenic Hypertension

If prolonged sympathetic neural activity can lead to structural changes in blood vessels thereby "fixing" the increased vascular resistance and arterial hypertension, such responses must be mediated through the blood vessels themselves. The mechanisms by which the sympathetic nerve impulse activity is transduced to effect changes in the arterial wall or of vascular receptors, however, is not understood. One possible mode of action could be through an effect on the metabolism of cyclic nucleotides in blood vessel walls. The cyclic nucleotide system might therefore be an important interface interposed between the sympathetic nerves and the blood vessels, since the levels of the cyclic nucleotides cyclic adenosine monophosphate (AMP) and cyclic guanosine monophosphate (GMP) appear to play a critical role in determining the tone of smooth muscle contractility and possibly cellular hyperplasia. Moreover, the metabolism and accumulation of these nucleotides may be altered by catecholamine neurotransmitters(36-38).

Recently, Amer(39) has demonstrated that the aortas from spontaneously hypertensive rats, or rats in whom hypertension is produced by prolonged stress, contain significantly lower concentrations of cyclic AMP than did their respective controls. It has been postulated that a reduction of cyclic AMP in blood vessels could be a common mechanism for the increased arteriolar resistance in both forms of hypertension. The reduced AMP levels resulted from increased activity of the degrading enzyme, phosphodiesterase.

Recently we(13, 14) have studied changes in the metabolism of cyclic nucleotides in aortas and hearts of rats in whom acute neurogenic hypertension was produced by NTS lesions to deter-

mine if similar changes in the metabolism of cyclic AMP occurred with this form of hypertension, if the changes occurred immediately, and if they could be attributed to the release of catecholamines and not to mechanical stresses secondary to the hypertension. In our study, tissues were obtained from rats two hours after the

CHANGES OF CYCLIC NUCLEOTIDE CONTENT WITH NTS HYPERTENSION

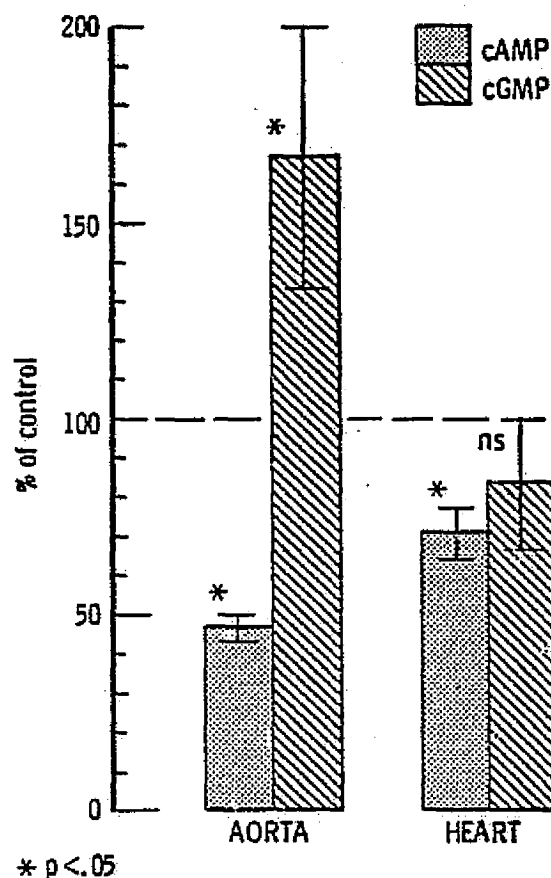


Figure 7-6. Changes in cyclic nucleotide content with NTS hypertension. Six to eight rats were killed two hours after NTS lesions were placed and they were hypertensive. Hearts and aortas were rapidly removed and frozen prior to assay for cyclic nucleotides.

Table 7-1
CHANGES IN CYCLIC NUCLEOTIDE METABOLISM IN AORTAS AND HEARTS
OF RATS WITH ACUTE NEUROGENIC HYPERTENSION

	Sham		Lesion	
	Aorta	Heart	Aorta	Heart
CYCLIC NUCLEOTIDE CONTENTS¹				
Cyclic AMP	0.38 ± 0.05	0.66 ± 0.08	0.18 ± 0.01*	0.47 ± 0.04*
Cyclic GMP	0.09 ± 0.03	0.06 ± 0.01	0.15 ± 0.03*	0.05 ± 0.01
Nucleotide "Index" ²	—	—	1.76†	1.17
ADENYLYL CYCLASE ACTIVITY³				
Basal	9.95 ± 1.12	33.00 ± 0.47	7.04 ± 0.52	29.79 ± 0.99
Isoproterenol	12.92 ± 1.23†	38.55 ± 2.38‡	7.00 ± 0.42	28.67 ± 3.08
Sodium Fluoride	14.67 ± 2.23†	42.47 ± 2.64‡	11.86 ± 1.12†	35.78 ± 1.83‡
GUANYLYL CYCLASE ACTIVITY³				
Basal	0.99 ± 0.24	4.75 ± 0.23	1.90 ± 0.25*	5.63 ± 0.46
PHOSPHODIESTERASE ACTIVITY⁴				
<i>Cyclic AMP as Substrate:</i>				
Total Activity	11.5 ± 1.5	60.8 ± 11.8	15.8 ± 4.5	62.7 ± 12.7
Percent of Low Km ⁵	2.6	2.2	3.8*	2.9*
<i>Cyclic GMP as Substrate:</i>				
Total Activity	12.7 ± 1.1	87.1 ± 2.0	13.6 ± 1.3	81.8 ± 4.0
Percent of Low Km ⁵	5.4	2.1	4.7	2.3
MEAN AORTIC BLOOD PRESSURE⁶				
	122.5 ± 2.1		172.6 ± 7.0§	

¹ pMoles/mg wet tissue; average of 4-10 determinations ± S.E.

² cyclic AMP sham — cyclic AMP NTS-lesioned

cyclic GMP sham — cyclic GMP NTS-lesioned

³ nMoles cyclic nucleotide formed/10 mg wet tissue/10 min (10-1). Average of 4-10 determinations ± S.E.

⁴ nMoles cyclic nucleotide hydrolyzed/5 mg wet tissue/10 min at 30° C. Average of 4-10 determinations ± S.E.

⁵ Calculated as described (24) from the data obtained at fifteen substrate concentrations.

⁶ mm Hg; average of six determinations ± S.E.

* Significantly different from sham-operated, $p < 0.05$; § $p < 0.01$.

† Significantly different from 1, $p < 0.05$.

‡ Significantly different from Basal, $p < 0.05$.

Courtesy of "H Ponte" (9).

placement of lesions. At this time, the hypertension is marked and maintained (Fig. 7-3).

During acute neurogenic hypertension, we found there was a significant decrease in the levels of cyclic AMP and an increase in

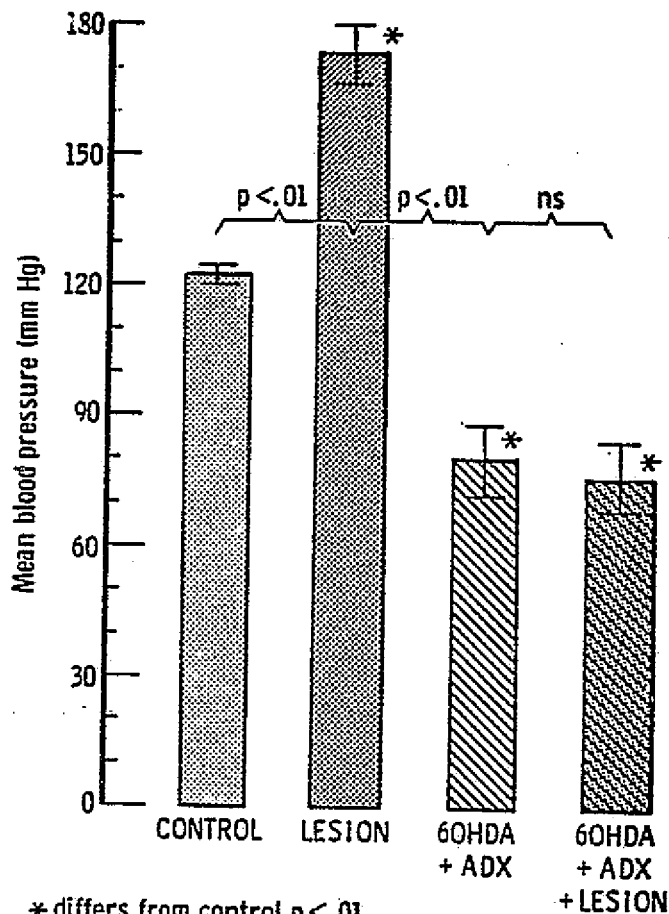


Figure 7-7A. Changes in mean aortic blood pressure two hours after NTS lesions, two hours after adrenalectomy (ADX) in animals treated with 6-OHDA (100 μ g/rat IV twenty-four hours prior to surgery) and after adrenalectomy and NTS lesions. Note that adrenalectomy and 6-OHDA lowers mean pressure below control(12) and blocks the pressor response to NTS lesions. Each group represents mean \pm SEM of six to eight rats. Bracket refers to differences between groups. * = differs from control $p < 0.01$.

cyclic GMP in the aortas of the lesioned compared to sham operated animals (Fig. 7-6, Table 7-1). In the heart, only cyclic AMP was significantly different from sham rats and was reduced. The decrease in cyclic AMP in aorta and heart was due to increased degradation of the nucleotide as a consequence of activation of the high affinity form of phosphodiesterase. The phosphodiesterase

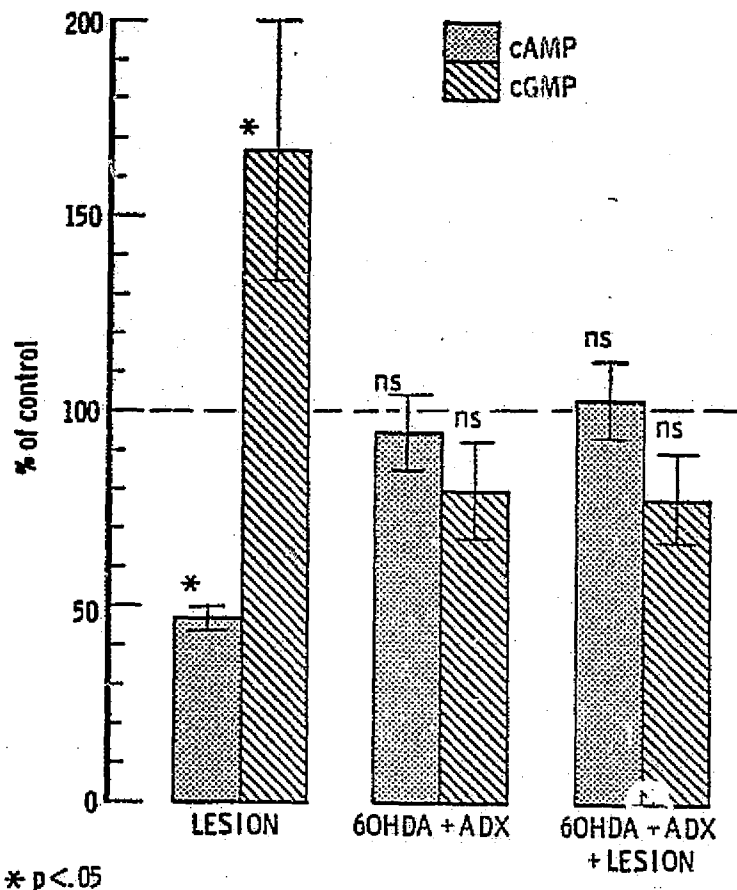


Figure 7-7B. Effects of NTS lesions on cyclic nucleotide content in aorta before and after 6-OHDA and adrenalectomy in rats whose arterial pressures are depicted in Figure 7-7A. Note that 6-OHDA and adrenalectomy alone do not alter the content of cyclic nucleotides but block the changes produced by NTS lesions. * = differs from control ($p < 0.05$). NS = not significant.

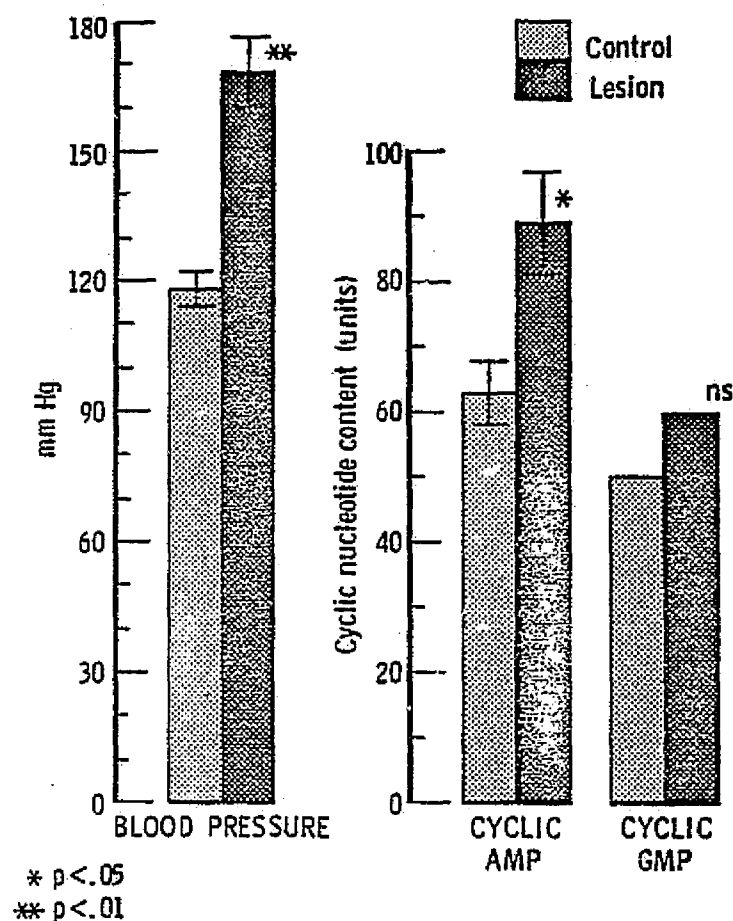
EFFECT OF AORTIC LIGATION ON CAROTID BLOOD PRESSURE AND MYOCARDIAL CYCLIC NUCLEOTIDE CONTENT

Figure 7-8. Effects of aortic ligation on mean arterial pressure in carotid artery and myocardial nucleotide content. Measurements are made two hours after ligation. Note that ligation increases intra-carotid pressure to a level comparable to that occurring with NTS lesions (Table 7-1). However, despite the elevation of pressure, myocardial cyclic AMP rises rather than falls (cf. Fig. 7-6 and Table 7-1). (Courtesy of "Il Ponte"[9]).

effect seemed restricted to that form of enzyme using cyclic AMP but not GMP as substrate (Table 7-I). While basal levels of adenylyl cyclase, the enzyme which catalyzes the biosynthesis of AMP, were unchanged, the enzyme could not be activated by the β -adrenergic agonist, isoproterenol (Table 7-I).

In contrast, the increase in GMP in the aorta appeared to be entirely due to increased synthesis of the nucleotide resulting from activation of the synthesizing enzyme guanylyl cyclase (Table 7-I).

The changes in cyclic nucleotide metabolism appeared to be secondary to catecholamine release and not to the hypertension *per se* since: (a) rats, previously treated with systemic 6-OHDA and in which the adrenal glands were removed just prior to placement of NTS lesions, did not develop the hypertension (Fig. 7-7A) nor did they demonstrate changes in the levels of the cyclic nucleotides in their aortas or hearts (Fig. 7-7B); (b) passive elevation of arterial pressure in the carotid artery and ventricle to levels comparable to those produced by NTS lesions by ligation of the aorta did not produce a fall of cardiac AMP (Fig. 7-8).

The results of this study, therefore, suggest that a very brief activation of sympathetic neurons can produce biochemical changes in the blood vessels and hearts of rat. These changes are in the same direction and of similar magnitude to those produced in animals by prolonged stress or with the genetically determined form of hypertension (SHR rats)(39). Since β -adrenergic receptor stimulation is usually associated with elevation of cyclic AMP and relaxation of smooth muscle while α -adrenergic stimulation and reduction of cyclic AMP would favor decreased relaxation and increased contraction of vascular smooth muscle(36-38), the direction of the changes would favor the production of increased peripheral resistance and hypertension. Conceivably, such changes of cyclic nucleotide metabolism may become fixed, leading ultimately to permanent changes in vascular resistance and blood pressure.

HYPERTENSION WITH HYPOTHALAMIC LESIONS: AH-HYPERTENSION

Recently we have discovered that lesions of another region of the brain can also produce fulminating hypertension in rat(14).

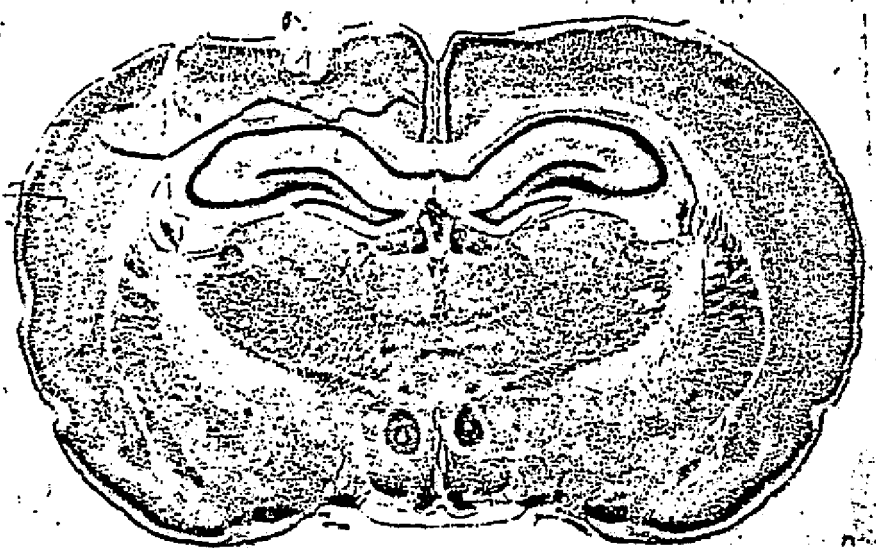


Figure 7-9. Bilateral electrolytic lesion of anterior hypothalamus in rat producing hypertension. Nissl stain.

Bilateral lesions of the anteromedial hypothalamus (Fig. 7-9) in animals anesthetized with halothane results in the development of increased arterial pressure within thirty to forty-five minutes after recovery from the anesthesia (Figs. 7-10, 7-11). The hypertension reaches a peak by ninety minutes and is maintained for several hours before the blood pressure begins to fall. The fall in blood pressure is associated with a fall of cardiac output, the development of progressive heart failure, pulmonary edema, and death. A record of typical animal is shown in Figure 7-10. We have termed this syndrome AH-hypertension.

Unlike NTS-hypertension, AH-hypertension is associated with a marked increase in motor activity (Figs. 7-10, 7-11) and also with hyperthermia. The motor activity, however, is not causal for the hypertension since the elevated blood pressure develops with the same latency and magnitude in paralyzed animals (Fig. 7-11). Similarly, it can be demonstrated that heating the animal to a comparable degree does not produce hypertension. At its peak, AH-hypertension, like NTS-hypertension, is associated with a marked

TIME COURSE OF CHANGES IN MOTILITY AND CARDIOVASCULAR RESPONSES AFTER AH LESIONS

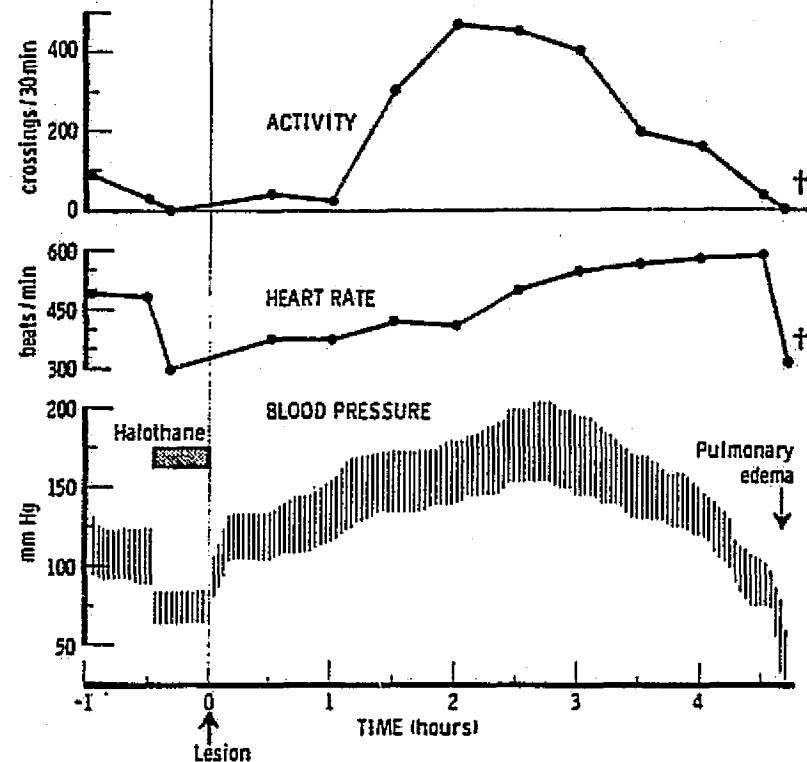


Figure 7-10. Time course of changes in arterial pressure, heart rate, and motility (as crossings of a photoelectric beam in an activity cage) following lesions of anterior hypothalamus.

increase in the total peripheral resistance, is reversed by α -adrenergic blockade, and is associated with a decrease in the cardiac output.

The striking feature of AH-hypertension distinguishing it from NTS-hypertension is that the elevated blood pressure, but not the motor activity, is entirely abolished by bilateral adrenalectomy, adrenal demedullation, or selective denervation of the adrenal glands performed just prior to the placement of brain lesions (Fig. 7-12). Thus, AH-hypertension is entirely the consequence of a selective release of adrenal medullary catecholamines. The ob-

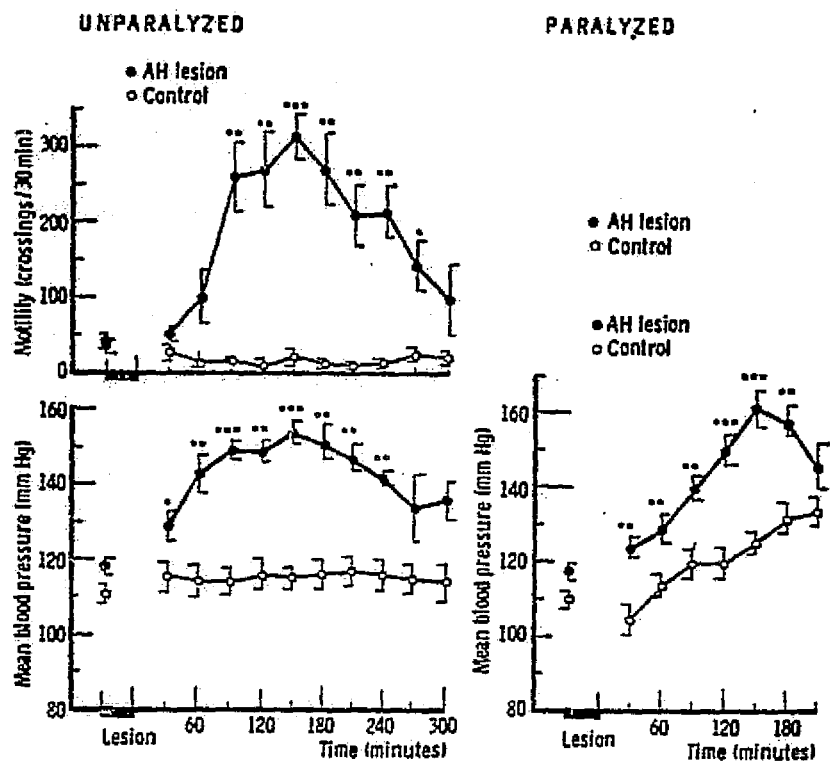


Figure 7-11. Effect of paralysis on development of hypertension following bilateral electrolytic lesions of anterior hypothalamus (AH).

Left panel: Changes in mean carotid arterial pressure and motility in unoperated control (open circles) and lesioned (solid circles) rats ($n = 10-20$) expressed as mean \pm SEM. Rats had carotid cannulas inserted under halothane anesthesia. After discontinuation of anesthesia, pre-lesion control values were obtained. Animals were reanesthetized (dark bar), lesions performed, and anesthesia discontinued. Note gradual development of hypertension and hyperactivity.

Right panel: Effect of paralysis on development of hypertension in unoperated control and AH-lesioned rats ($n = 12-14$). After placement of lesion and insertion of tracheal cannula, animals were paralyzed with d-tubocurarine (0.4-0.8 mg/kg IV) and artificially ventilated on a gas mixture of 50 percent O_2 and 50 percent N_2 . Blood gases were normal. Note that hypertension develops in absence of motor activity. Significance of differences from control: ** = $p < 0.01$; *** = $p < 0.001$.

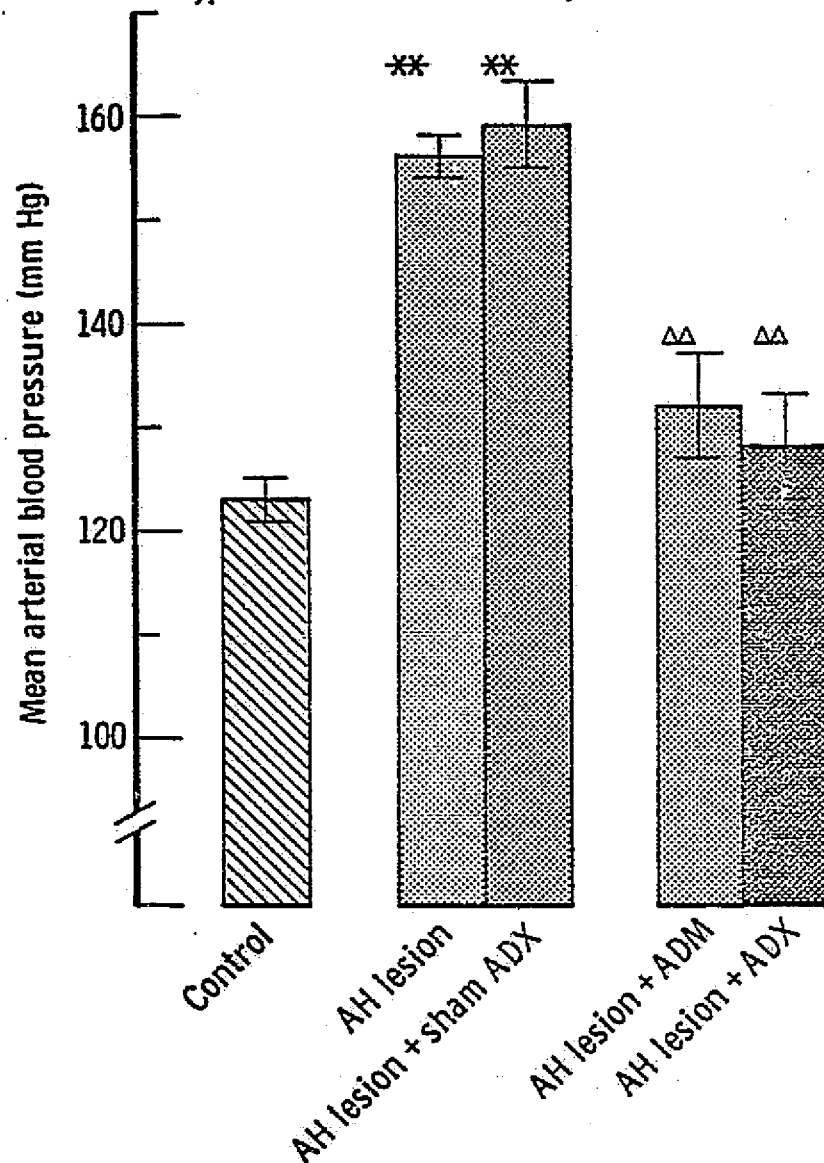


Figure 7-12. Effects of bilateral adrenal demedullation (ADM) or denervation (ADX) on hypertension produced by lesions of AH. Note that demedullation and total adrenal denervation abolished hypertension. Blood pressure measured two hours after lesions and adrenal surgery. Results expressed as mean \pm SEM for six to eight animals. ** — differs from control ($p < 0.001$); $\Delta\Delta$ — differs from control and AH lesions ($p < 0.001$).

servation is of interest and indicates that structures either originating in or passing through the region of the anteromedial hypothalamus exert a tonic inhibitory effect on adrenomedullary secretion of catecholamine. Moreover, they also indicate that within the brain, the adrenal medulla may have a representation in part quite separate from that of the remainder of the sympathetic system.

The finding that lesions of the anterior hypothalamus produce hypertension is not surprising in view of the fact that electrical stimulation within anterior hypothalamus has been shown to result in an inhibition of arterial pressure and a decrease in the heart rate(21,22). In cat, the region lying at about the same rostrocaudal level, but presumably more dorsolaterally situated in anterior hypothalamus, has been identified as an important sympatho-inhibitory region(22, 23). Whether or not our lesions in the rat interrupt the same system remains to be determined. However, it is evident that hypertension can be produced in rat not only by destruction of sympatho-inhibitory pathways in the lower brainstem but in rostral hypothalamus as well.

Some years ago, Patton and his associates(40-42) described the production of pulmonary edema in association with hypermotility and hyperthermia by lesions of AH in rats. Unfortunately blood pressure was not measured. It is probable that Patton produced a syndrome comparable to the one we see in the rat. In both studies lesions placed in roughly comparable positions in the brain resulted in hyperactivity, hyperthermia, and pulmonary edema. However, our finding that these animals are markedly hypertensive suggests the etiology of the pulmonary edema, namely that it is due to progressive heart failure resulting from ventricular overload.

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DISCUSSION

Dr. P. M. Gootman (State University of New York, Downstate Medical Center): Maire and Patton found that bilateral

lesions in the preoptic area (slightly more anterior to your anterior hypothalamic locus) also produced pulmonary edema.*

I wonder whether their site and yours might both inhibit other sites in the central nervous system, i.e. what area or areas are released from inhibition. The destruction of these areas would be expected to prevent the occurrence of pulmonary edema.

Dr. Reis: We think this is the same model as that of Patton and Maire; they just never measured blood pressure. We believe that the pulmonary edema is a terminal event and not a consequence of a selective release of some edemagenic area of the brain. They found that pulmonary edema could be blocked by treatment with reserpine or by transection of the splanchnic nerves which, of course, will denervate the adrenal glands. It is obvious that other areas of the brain are involved in the syndrome as a release phenomena but at the present we do not know whether the effect is due to damage of cells or fibers of passage in the region nor do we know what nuclei below the lesion are critical for the expression of the hypertension.

Dr. Gootman: Suppose you made an anterior hypothalamic lesion and an NTS lesion?

Dr. Reis: We haven't done that; such an experiment would be interesting.

Dr. Gootman: Thank you.

Dr. A. Zanchetti (University of Milan, Italy): These are very beautiful experiments, and a lot of questions come to my mind. The first is about the remarkable difference between the dramatic rise in arterial pressure you got by lesion of NTS and the very moderate one that is observed when baroreceptive afferents are cut at the neck. This is not only a matter of anesthesia, as a very mild hypertension is ob-

*Maire, F. W. and Patton, H. D.: Hyperactivity and pulmonary edema from rostral hypothalamic lesions in rats. *Am J Physiol*, 178:315, 1954.

Maire, F. W., and Patton, H. D.: Neural structures involved in the genesis of "preoptic pulmonary edema," gastric erosions and behavior changes. *Am J Physiol*, 184:345, 1956.

served in chronic animals subjected to sino-aortic denervation. This was apparent from the figure taken from one of my papers that John Dickinson kindly showed during his review (Chap. 6). This has been confirmed by others and quite recently in Dr. Guyton's laboratory. So I am asking myself whether this difference reflects mainly a more complete baroreceptor deafferentation achieved by NTS lesion, where all these afferents converge. Of course, cutting the carotid sinus and aortic nerves in the neck leaves a large number of baroreceptive afferents in the vagi, but to my recollection, Guyton's group did cut a large number of vagal fibers as well. A second possibility is that you are destroying in the solitary tract nucleus something more than only baroreceptive endings. A final possibility is the time course. In chronic experiments, sinoaortic denervation is performed under deep and prolonged anesthesia, which you say blocks the dramatic rise you observed in your unanesthetized rats. Have you tried to do your lesions under barbiturate anesthesia lasting for a few hours, and then see if the dramatic and killing hypertensive crisis again occurs, though delayed, when the anesthetic fades away?

Dr. Reis: I think you have raised a very important point as to why we get such fulminating hypertension by NTS lesions when others have shown in rat and different species that with sinoaortic denervation alone the hypertension is not fulminant. We believe NTS lesions also damage afferent fibers from not only arterial baroreceptors but including, for example, mechanoreceptors from the heart, all of which produce a common depressor reflex. Thus NTS lesions will interrupt every kind of peripheral input particularly those from the vagus which result in a reflex inhibition of sympathetic tone. In addition, the lesion may impair central neurons or projections into NTS which also facilitate depressor responses. The barbiturate experiment has not been done.

Dr. P. Kezdi (Cox Heart Institute, Dayton): This is a comment to Dr. Zanchetti's comment. Of course you know that it is difficult to produce chronic hypertension by sinoaortic de-

afferentation. This requires a multiple stage operation because using the Thomas technique in one stage causes the animals to die in acute heart failure and pulmonary edema. This would correspond to the concept of complete central sinoaortic deafferentation by lesion of the nucleus solitarius in these experiments. Would it be possible to produce chronic animal hypertension in your model by ameliorating the initial, marked elevation of the blood pressure by partial sympathetic blockade or by doing the lesion on one side first and then later on the other side?

Dr. Reis: We have tried this. However, there is one consequence of the lesion which may be particularly dangerous in the rat. One of the consequences of the NTS lesions is that they impair afferent impulses arising from the lung. These animals develop a severe atelectasis toward the termination of their illness, we believe, not only because they are in cardiac failure but also because of primary pulmonary dysfunction. Acute pulmonary failure, however, does not cause hypertension in the acute stage. We have measured blood gases and they are normal. But later on, because of development of atelectasis, the animals do not fare well. So the rat has certain limitations in this regard for production of chronic hypertension.

Dr. M. J. Brody (University of Iowa): We have found that intracisternal administration of 6-hydroxydopamine abolishes the central pressor effect of angiotensin produced by its intravertebral administration. When we looked at catecholamines in the brain we found that there was no brain stem depletion although there was depletion rostral to and below the brain stem. We concluded that 6-hydroxydopamine might not have been depleted because there is primarily an adrenergic cell body rather than a neuronal population in brain stem. We thought, therefore, the increase in pressure produced by angiotensin might have involved activation of a high spinal neural tract. I wonder if you have looked at brain stem depletion in your experiments?

Dr. Reis: Yes. We didn't find depletion of norepinephrine in the brain stem with the dose used. There are several problems

with 6-hydroxydopamine. First of all, there are many terminals as well as fibers in the brain stem so I don't agree with your interpretation completely. Secondly, we have found other reasons why 6-hydroxydopamine may not alter brain stem amines. We have recently found that in the rat with careful intracisternal injection of drugs or dyes there is little penetration into the floor of the fourth ventricle. Now this may be a species variation but I think one has to be very careful in assuming diffusion by intracisternal administration. By this route the drug is not introduced into the ventricular system but the subarachnoid one, thereby allowing for a preferential transport either over the dorsal surface of the brain or down into the spinal cord. However, I would agree that the major site of action is in the spinal cord region. This would be in agreement with the conclusions of Chalmers and Reid.

Dr. F. R. Calaresu (University of Western Ontario, London): I have no question now, just a comment. Merva Cottle and I placed some very small lesions in NTS in 1963, and she has just finished looking for degenerating fibers with the Nanta and Finkheimer techniques. She has shown very clearly that most of the output of the NTS goes caudally, and unfortunately we did not keep the spinal cords so we cannot say that it terminates in the intermediolateral nucleus. This is interesting information to put together with the role of the excitatory hypothalamic input to intermediolateral nucleus described by Orville Smith which keeps the neurons in an excitatory state. Under ordinary circumstances these neurons are kept under check by the NTS input and when the NTS input is removed, this hypothalamic excitatory input is released again.

Dr. Reis: Conceivably. As one of my friends once said, "The nervous system is all excitation and inhibition." However, the effect of NTS lesions cannot be attributed to destruction of a descending pathway alone since decerebration abolishes the responses. Thus rostral regions appear to be released. The pathways involved are unknown. However, the fact that ascending projections of baroreceptors in the carotid

sinus follow a multisynaptic course may explain the exquisite sensitivity of NTS hypertension to anesthetics since by and large the more synapses, the greater the sensitivity of a pathway to anesthetic agents.

Dr. Calaresu: Right. It's too bad you didn't quote the work we did on hypothalamic single units affected by baroreceptors and chemoreceptors.

Dr. Reis: But you have, right?

Dr. C. J. Dickinson (University of London, England): Just a brief clinical comment. I think this is an entirely plausible model of one situation which I have certainly seen in one patient with some acute brain stem lesion—unfortunately, I don't remember exactly where it was—who had an exquisitely phentolamine-sensitive hypertension and in whom there was a vast excess of catecholamines in the urine. Such cases have been described following acute lesions in man, and I think you may have a very exact animal model of that situation.

Dr. Reis: A caveat! In the literature there are a small number of cases in which arterial hypertension has resulted as a consequence of a lesion of the fourth ventricle. However, the lesion is usually a mass lesion and is pressing on the floor of the fourth ventricle. Now, the regions of the brain stem which mediate increase in blood pressure to intracranial pressure (the Cushing reflex) have been shown by Doba and myself to be very different from the NTS. The receptive region lies in a nuclear group lying just beneath the floor of the fourth ventricle in the dorso-lateral reticular formation and is quite distinct from NTS. So I think the kind of hypertension people see in patients with cerebellar tumors or acoustic neuromas is different. The one hypertensive state due to brain stem lesions is that which is seen in acute encephalitis or poliomyelitis. However, even in these cases the distribution of the lesions seems to fall not in NTS but in the paramedian reticular area lying beneath. However, this, I think, is the one clinical state of hypertension with lesions. The reason may be that bilateral lesions are required to produce the hypertension. Bilateral lesions of

the brain stem in this region, however, are not usually compatible with life.

Dr. C. Ferrario (Cleveland Clinic): I want to comment that NTS hypertension from a hemodynamic point of view is similar to that caused by infusion of angiotensin into the vertebral artery circulation; this response probably involves the area postrema which is in close proximity to the NTS nuclei. I wonder whether maybe angiotensin can chemically simulate a similar response.

Dr. Reis: Two comments. First, it's clear that our lesions spare the area postrema and thus this nucleus is not responsible. And so the relationship of the area postrema to the angiotensin response is very interesting. I'm just a little bit skeptical, however, that the area postrema alone mediates the angiotensin response since the nucleus solitarius is so close to the area postrema that some damage of NTS may occur as a secondary effect, possibly by damaging dendrites extending from NTS into the region. Another interesting feature is that there is a very rich noradrenergic innervation of both areas and it is known that angiotensin releases catecholamines. Thus one possibility is that catecholamine metabolism may in some way mediate this effect of the angiotensin in the area postrema. However, as I indicated, it cannot explain the NTS hypertension entirely.

Dr. W. M. Manger (Institute of Rehabilitation Medicine, New York University Medical Center): I'm sorry but I didn't grasp the implication of your cyclic nucleotide studies, how they fitted into the hypertensive picture. One time you had lower values of cyclic AMP and GMP, that was in the aorta, and then you had just lower cyclic AMP in the heart.

Dr. Reis: Yes, I ran through that rather quickly. It's been shown in a number of systems that lowering of cyclic AMP in smooth muscle, gallbladder, stomach, heart, striated muscle, and blood vessels is always associated with stimuli which result in vasoconstriction. And likewise, an elevation of cyclic GMP seems to be also related to this state. Amer showed several years ago that the blood vessels and hearts of animals that were hypertensive, either spontaneously

hypertensive rats or rats made hypertensive by chronic stress, had cyclic AMP levels which were low and he associated this with increased resistance. Now what we've done is to repeat this study with an acute sympathetic stimulation to vessels and heart. We have found a similar reduction of cyclic AMP in the blood vessels with an elevation of GMP and that this effect can be abolished by treating with 6-hydroxydopamine and adrenalectomy, thereby eliminating catecholamine release and the hypertension. And we also were able to show it is not a consequence of mechanical distortion produced by aortic ligation.

Dr. Manger: But isn't cyclic AMP associated with the energetics of relaxation, so that impairment of relaxation would result from decreased cyclic AMP?

Dr. Reis: Yes. Thus a *decrease* should be in the direction of a diminished relaxation, i.e. increased constriction and resistance and thus hypertension.

THE TRIGEMINAL DEPRESSOR RESPONSE:
A NOVEL VASODEPRESSOR RESPONSE ORIGINATING FROM
THE TRIGEMINAL SYSTEM

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SUMMARY

Electrical stimulation within discrete sites of the spinal trigeminal complex in anesthetized or decerebrated rabbits results in arterial hypotension, often over 50 mm Hg, bradycardia of up to 60 beats/min, apnea, and gastric hypermotility, collectively termed the trigeminal depressor response (TDR). The threshold for the TDR is ≤ 10 μ A and is graded up to 3-6 times threshold. It can only be elicited by trains of stimuli of low frequency (0.5-20 Hz); at 50 Hz the response disappears or becomes pressor. The bradycardia is only abolished by bilateral vagotomy combined with β -adrenergic blockade, and thus, results from combined excitation of cardio-vagal and inhibition of cardiac sympathetic nerves. The hypotension is unassociated with changes in cardiac output, does not change after blockade of the bradycardia, but disappears after α -adrenergic blockade and hence is entirely attributable to inhibition of ongoing sympathetic vasoconstrictor nerve activity. Below threshold stimulation the TDR can only be elicited from the root entry zone of the Vth nerve, from dorsal portions of the spinal tract of the Vth nerve, and to portions of the nucleus of the spinal tract, notably the nucleus caudalis. A TDR of slightly reduced magnitude can also be elicited by low frequency stimulation of numerous branches of the Vth nerve arising from all three divisions and including the supra- and infra-

orbital, the inferior alveolar, and lingual nerves.

Bilateral electrolytic lesions of the nucleus tractus solitarii at the obex, with complete abolition of baroreceptor reflexes from carotid sinus and aortic depressor nerves, fails to alter the TDR elicited from brain or from branches of the Vth nerve, nor vasodepressor responses elicited by electrical stimulation of the central ends of the IXth and Xth cranial nerves transected distal to the branches of baroreceptor nerves. In contrast, caudal lesions of the trigeminal complex abolish the TDR elicited from brain and Vth nerve and the vasodepressor responses from the IXth and Xth nerves, without altering baroreceptor reflexes.

We conclude that the TDR represents a heretofore recognized vasodepressor response dependent upon the spinal trigeminal complex which is at least in part anatomically distinct from pathways subserving arterial baroreceptor and somatic vasodepressor reflexes. The TDR can be reflexly elicited from widely distributed but yet unidentified receptors innervated by branches of the Vth and of the IXth and Xth cranial nerves other than those innervating arterial baroreceptors. It is of unknown function, but may be related to pain mechanisms.

INTRODUCTION

We have recently discovered that electrical stimulation within portions of the spinal trigeminal complex* of anesthetized or decerebrated rabbits will elicit a marked fall of arterial pressure and bradycardia.²⁸ We have termed the response the trigeminal depressor response (TDR) with reference to its presumed site of integration in the spinal trigeminal complex. The TDR appears to be a unique depressor response, previously unrecognized, and differing from other autonomic reflexes which are well established as being mediated through the trigeminal nerves, including the diving reflex,³ the response to noxious stimuli of oral-facial mucosa (the nasopharyngeal reflex)^{4,52} and the response to pressure on the eyeball⁵ (oculocardiac reflex; Aschner's reflex).

In the present study we have sought to: (a) further characterize the TDR by examining in greater detail its neurophysiological and cardiovascular characteristics; (b) map its distribution throughout the spinal trigeminal complex; (c) determine if it can be reflexly initiated from the trigeminal nerve, and finally, (d) examine its relationship to other depressor responses, particularly those elicited from arterial baroreceptors and other receptors innervated by the IXth and Xth cranial nerves.

*In this paper the spinal trigeminal tract and its nucleus are collectively called the "spinal trigeminal complex".

METHODS

Preparation of animals

Experiments were performed on 77 adult New Zealand white rabbits of both sexes weighing 3.0-4.5 kg. Sixty animals were anesthetized by urethane (1-1.5 g/kg., i.v.). The remainder were decerebrated at the supracollicular level while anesthetized with halothane (3% in 100% O₂) blown over the nose through a face mask. After insertion of tracheal, arterial, and venous cannulas and, in selected experiments, probes and transducers for recording cardiac output, respiration, or gastric motility, the animal was placed in a stereotaxic frame with the head flexed to 45°. A polyethylene arterial catheter (P.E. 160 i.d. 0.45") was inserted in the femoral artery to record arterial pressure. Except for 5 experiments in which respiration was monitored, the animals were then paralyzed with gallamine triethiodide (5 mg/kg, i.m.) and artificially ventilated by a Harvard respirator pump on a gas mixture of 50% O₂ and 50% N₂. Body temperature was maintained at 37°C by a thermostatically regulated heating pad connected to a rectal thermistor probe.

Measurement of cardiovascular, respiratory and gastric activities

Instantaneous and mean arterial pressure and heart rate were continuously recorded in all experiments. Arterial

pressure was recorded from the abdominal aorta by a polyethylene catheter inserted through the femoral artery and connected to a Statham P23 Db transducer. Heart rate was computed from the arterial pressure pulse by a tachometer (Beckman 9857). In 5 animals, instantaneous cardiac output (minus coronary flow) was measured through an electromagnetic flow transducer (Narco C series) attached after thoracotomy to the root of the ascending aorta under positive-pressure respiration. The flow transducer was connected with a flowmeter system (Narco, RT-400). Mean blood flow was recorded through an integration circuit built into the flowmeter and having a time constant of 1 sec. Total peripheral resistance was calculated as: arterial pressure/cardiac output..

In 5 animals, respiration was measured by a polyvinyl balloon catheter implanted in the thorax between the lungs and the pleura. The balloon catheter was inflated by about 5 ml of water and connected to a Statham P23 Db transducer to detect changes in the intrathoracic pressure.

In 4 animals, gastric motility was measured by a latex balloon catheter placed in the stomach through a mid-abdominal incision. The balloon was inserted into the stomach through a small hole in the greater curvature near the pylorus and then filled with 150-200 ml of water warmed to 37°C. The balloon was connected through a catheter to a Statham P23 Db transducer to record intragastric pressure. All transducers were connected to channels of a polygraph (Beckman Dynograph Recorder, 504A).

Stimulation and lesions of the brainstem

The floor of the fourth ventricle was exposed by removing the occipital bone between the atlanto-occipital membrane and the nuchal ridge. In some experiments the vermis of the caudal cerebellum was removed by gentle aspiration for subsequent penetration of the medulla by the electrodes.

The area of the spinal trigeminal complex was explored stereotaxically. Electrodes were inserted perpendicular to the brainstem directly visualized through a Zeiss dissecting microscope. The antero-posterior and lateral coordinates of each electrode tract were determined with reference to the obex. The vertical reference was to the surface of the brain at the point of entry. Exploration was assisted by reference to stereotaxic atlases of rabbit brain.^{33,53} Tracks were explored in 250 μ m steps. For subsequent identification of stimulation sites, each track was marked at two spots by passing 20 mA current for 30 sec in order to deposit iron.

Stimulation of the spinal trigeminal complex and adjacent areas was done through electrodes made of 0.006 inch Teflon-coated stainless steel wires bared at the tip for 50 μ and carried in no. 28 stainless steel hypodermic tubing. Electrical stimuli were square-wave pulses of 0.5 msec duration, delivered to the animal from a Tektronix pulse generator through an r.f. stimulus isolation unit. The stimulus current, measured by passing the stimulus across a 10-ohm resistor, was amplified by a Tektronix 122 preamplifier and displayed on a cathode ray oscilloscope and monitored through-

out the experiment. Stimulation was monopolar, the anode being a silver plate attached to the neck muscle.

In 12 experiments lesions were made within the nucleus tractus solitarii to abolish the arterial baroreceptor reflex. Lesions were placed by passing a d.c. anodal current of 2-4 mA from a constant current source for 12-40 sec through an electrode identical to the stimulating electrode, but with a tip exposure of 500 μ . In each animal 2 bilateral lesions were made, one pair rostral and the other caudal to the obex. The coordinates for the rostral lesions were 0.4 mm anterior to the obex (A 0.4), 1.3 mm lateral to the midsagittal line (L 1.3) and 0.5 mm below the dorsal surface of the brainstem (D 0.5). The coordinates for the caudal lesions were 0.6 mm posterior to the obex (P 0.5), L 0.6 and D 0.5.

In 8 experiments the spinal trigeminal tract and the underlying subnucleus caudalis of the trigeminal spinal nucleus were mechanically destroyed by a knife or scaled tweezer with the aid of stereotaxic apparatus. Destruction was made 2-3 mm caudal to the obex extending laterally from 2 mm to the lateral end with the depth of about 2 mm.

Stimulation of peripheral nerves

Various cranial nerves or their branches were dissected free of surrounding connective tissue and transected. The proximal end was placed across a pair of platinum wire electrodes spaced 2-5 mm. Although the nerve preparation was

left in air, care was taken to keep the preparation properly moist. Electrical stimuli were square-wave pulses of 0.5 msec duration delivered to the animal from a pulse generator (Tektronix) through an isolation unit. The stimulus frequencies usually were between 5 to 50 Hz and the stimulus currents ranged from 50 to 1000 μ A.

Branches of the trigeminal nerve were prepared for stimulation as follows: the supraorbital nerve was identified at its exit from the orbit through the supraorbital groove (5 experiments). After enucleation, the nerve was isolated within the orbit. The infraorbital nerve was identified either at its exit through the infraorbital foramen or within the nasal cavity (4 experiments). In the latter case the nasal bridge was incised and the alar cartilage was removed to expose the nerve. The inferior alveolar and lingual nerves were exposed by removing part of the mandibular body and separating the medial and lateral pterygoid muscles. The inferior alveolar nerve was identified at its entry to the mandibular canal (4 experiments). The lingual nerve was found anterior to the inferior alveolar nerve (4 experiments).

In another 8 experiments the facial (VIIth), glossopharyngeal (IXth), and vagal (Xth) nerves were stimulated. The facial nerve was dissected by opening the facial canal along the posterior margin of the external ear (3 experiments). The glossopharyngeal nerve was dissected in the neck distal to branching of the carotid sinus nerve (4 experiments).

The vagal nerve was exposed in the neck distal to branching of the aortic nerve (4 experiments).

Histological examination

At the termination of the experiment, the animal was perfused with saline followed by 10% formaldehyde and 1% potassium ferro- and ferricyanide in order to identify the tips of the stimulating electrodes by the Prussian blue reaction.¹³ The brain was then fixed frozen and sectioned at 50 or 100 μ . The sites of iron deposition were identified and related to histological structures before and after staining the sections for cell (Nissl) or fibers (Weil).

RESULTS

A. General Characteristics of the Trigeminal Depressor Response (TDR)

Low frequency stimulation of the spinal trigeminal complex (Fig. 1) evoked a large and abrupt fall in systolic and diastolic arterial pressures and heart rate: the TDR. The fall in arterial pressure, often greater than 50 mmHg, occurred with a latency of less than 3 sec, was sustained during stimulation, and rapidly recovered. The bradycardia, often greater than 60 beats/min, had its onset within the first 3 sec. In contrast to the fall in arterial pressure, however, the bradycardia was usually not sustained and gradually returned toward control levels during the stimulation period.

That the response is anatomically specific for the trigeminal complex, and not a consequence of stimulus spread, was demonstrated in 3 ways: Firstly, when the brainstem was explored in 0.5 mm steps with low stimulus currents, a response was only evoked from within the spinal trigeminal complex (Fig. 1). Secondly, the lowest stimulus currents along a positive tract which elicited detectable (threshold) responses were sharply demarcated and restricted to the trigeminal complex (Fig. 2). The lowest threshold current was usually 10 μ A or less. Thirdly, lesions placed

at sites of lowest stimulus thresholds abolished the response even when the stimulus intensity was increased.

B. Localization of Active Sites

A systematic exploration for sites of lowest threshold eliciting the depressor response was undertaken. The area of the spinal trigeminal complex was explored in 250 μ steps. At each point the brain was stimulated with a 12 sec train of square-wave pulses at a frequency of 5 Hz and an intensity of 100 μ A. At sites from which a fall of arterial pressure and bradycardia were elicited, the threshold was determined by varying the stimulus current.

Fig. 3
here

Figure 3 summarizes the localization of positive and negative loci as well as the sites of lowest threshold for eliciting the depressor response from 80 electrode tracks explored in 22 animals. These are projected onto nine coronal sections of the medulla extending from the level just rostral to the entry of the trigeminal nerve root down to the upper cervical cord (Fig. 3).

Several features of the distribution of the TDR should be noted. First, the sites of low threshold responses (≤ 10 μ A) are restricted to selective portions of the trigeminal complex, including the entering roots (Fig. 3B), and the entire course of the spinal tract (Fig. 3c-i). In the tract, particularly rostrally, the response primarily lies in the dorsal portions. Second, positive sites are found

within two caudal nuclear subdivisions of the nucleus of the spinal tract of V, the nuclei caudalis and interpolaris.^{10,39} On the other hand, no responses were elicited from within the principal sensory, the mesencephalic and the motor nuclei of the Vth nerve (Fig. 3b), even with stimulus currents up to 200 μ A. Third, the response was not elicited at sites rostral to entering roots of the trigeminal nerve (Fig. 3a). Fourth, no, or very small, responses were ever elicited at sites medial or lateral to the trigeminal complex, nor from within portions of the nucleus tractus solitarii (Fig. 3d-f, NTS) corresponding to the course of entering roots of the IXth and Xth cranial nerves. Thus, it would appear that the response is due to stimulation of fibers in the spinal tract of the Vth nerve, many of which are probably of trigeminal nerve origin and which traverse the spinal tract to either synapse or emerge through the caudally placed sensory nuclei.

C. Elicitation of TDR by electrical stimulation of branches of Vth nerve

Various branches of the branches of the trigeminal nerve representing the three major divisions were stimulated (Fig. 4). Stimulation of the supraorbital (Fig. 4a), infra-orbital (Fig. 4b), inferior alveolar (Fig. 4c) and lingual nerves (Fig. 4d) always elicited hypotension and bradycardia. The responses obtained from the supra- and infraorbital

Fig. 4
here

nerves were greater than those elicited from the inferior alveolar and lingual nerves. The maximal depressor response evoked from a peripheral branch was usually between 14 and 50 mmHg, a value somewhat smaller than that obtained from stimulation within the brain, yet substantial. The cardiovascular responses to stimulation of branches of the Vth nerve were blocked by an ipsilateral lesion placed within the spinal trigeminal tract near the obex. Thus, it appears that the TDR may also be reflexly elicited from branches of the trigeminal nerve.

D. Effect of stimulus frequency and intensity on the TDR elicited centrally or from the trigeminal nerve

The TDR was optimally elicited by electrical stimulation of brain or peripheral nerves at low frequencies (Fig. 5a); the optimal frequency was between 5 and 10 Hz both centrally and peripherally. The lowest frequency for eliciting the TDR centrally was about 0.3-0.6 Hz and slightly higher peripherally at 0.5-1 Hz. As the stimulus frequency was increased, both centrally and peripherally, the magnitude of the depressor response declined. At frequencies higher than 50 Hz, it sometimes converted to a pressor response.

The threshold of the TDR elicited by a 12 sec stimulus train at the optimal frequency at an active brain site was 10 μ A or less. The response was graded with regard to stimulus intensity (Fig. 5b) up to stimulus intensities 3 to 6 times threshold.

E. Mechanism of Cardiovascular Changes in the TDR

1. Heart Rate

Fig. 6
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The bradycardia of the TDR was attenuated but not abolished by bilateral cervical vagotomy (Fig. 6b) or intravenous injection of 2 mg/kg of atropine sulphate. Vagal blockade reduced the magnitude of the fall in heart rate occurring at the onset of stimulation. β -adrenergic blockade, (propranolol, 2 mg/kg, i.v.), also attenuated but did not abolish the bradycardia (Fig. 6e). For complete elimination of the bradycardia, combined vagal and sympathetic blockade (Fig. 6c) were required. Thus, the bradycardia associated with the TDR is due to combined excitation of cardiac vagal and inhibition of cardiac sympathetic nerves.

2. Arterial Pressure Response

Fig. 7
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The hypotension of the TDR was not due to a fall of cardiac output since cardiac output was essentially unchanged during the response (Fig. 7a-c), and the hypotension persisted after bradycardia was abolished by vagotomy combined with β -adrenergic blockade (Fig. 6c). On the other hand, the hypotension (but not the bradycardia) was abolished by α -adrenergic blockade with phentolamine (1 mg/kg, i.v.), either alone (Fig. 7e) or in combination with propranolol (Fig. 7c).

3. Changes in Respiration and Gastric Motility

In rabbits breathing spontaneously, low intensity electrical stimulation of the spinal trigeminal tract and its nucleus elicited tachypnea with decreased tidal volume. Slightly stronger stimulus currents produced expiratory apnea (Fig. 8a). The respiratory response appeared within 2 sec after the onset of the stimulus and prior to the fall in arterial pressure, thereby demonstrating that they were not reflex.

Electrical stimulation of the spinal trigeminal tract also elicited increased gastric contractions. These began 15 to 20 sec after the onset of stimulation and were maximal 10 sec later (Fig. 8b). Gastric motility was partially reduced by transection of the ipsilateral or contralateral vagus. Bilateral vagotomy was required for completely abolishing the gastric response.

The most sensitive sites for elicitation of changes in respiration and gastric motility were identical to those eliciting the cardiovascular responses. At the most sensitive locus, the threshold for the gastric contraction was slightly higher than that for the circulatory or respiratory changes which shared common thresholds.

F. Anatomical Relationship Between TDR and Arterial Baroreceptor Reflexes

There is a striking similarity between the TDR and arterial baroreceptor reflexes: Both consist of bradycardia, and hypotension,¹⁹ apnea,¹⁸ and gastric hypermotility.^{21,27} Since it is known that baroreceptor reflexes from the carotid sinus and aortic depressor regions are mediated by the nucleus tractus solitarii (NTS), and that bilateral electrolytic lesions of NTS will abolish baroreceptor reflexes,^{15,35} we sought to establish, in 5 animals, if lesions of NTS could also impair the TDR.

Fig. 9
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A typical experiment is illustrated in Fig. 9. The baroreceptor reflex was assessed by eliciting a reflex bradycardia by administration of a pressor dose of norepinephrine (NE). In this, and all other experiments, lesions of NTS completely abolished the baroreceptor reflex without altering the TDR elicited by electrical stimulation of the nucleus caudalis of the spinal trigeminal tract. These results indicate that at least some of the central pathways for the TDR are independent of those mediating reflex responses from arterial baroreceptors.

G. Effects of Lesions of the Spinal Trigeminal Complex on Cardiovascular Responses, Other Than Baroreceptor, Elicited from the IXth and Xth Nerves

Stimulation of receptors innervated by branches of the IXth and Xth nerves (other than the carotid sinus and aortic depressor nerves) can elicit vasodepressor responses^{36,41} similar to the TDR. Since branches of the facial (VIIth), glossopharyngeal (IXth) and vagus (Xth) nerves project into the spinal trigeminal tract^{2,9,10,24,25,48}, we sought to determine if some of those depressor responses are also mediated by the spinal trigeminal tract, and also, if they are independent of baroreceptor mechanisms.

Therefore, in 6 rabbits, we analyzed the effects of lesions of the spinal trigeminal tract and/or NTS on cardiovascular reflexes elicited by electrical stimulation of the central end of the transected IXth and Xth nerves. Stimulation of the central end of VII did not elicit any responses.

g. 10
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The results of a typical study is illustrated in Fig. 10. Low frequency (5-20 Hz) stimulation of the vagal trunk distal to the junction of the aortic nerve (AN) elicited hypotension and bradycardia (Fig. 10b) comparable to that elicited by electrical stimulation of the aortic nerve itself (Fig. 10a). The response from the vagal trunk, but not from the aortic nerve, was eliminated by destruction of the dorsolateral part of the ipsilateral lower brainstem, including most of the spinal trigeminal complex (Fig. 10c and 10d). In contrast, bilateral electrolytic lesions of NTS abolished the aortic depressor (Fig. 10e), but not the vagal depressor response (Fig. 10f).

In comparable experiments low frequency (5-20 Hz) stimulation of the glossopharyngeal nerve, distal to the junction of the carotid sinus nerve, also caused a depressor response with a bradycardia. Abolition of the reflex bradycardia, elicited by stimulation of carotid arterial baroreceptors by NTS lesion, failed to impair the depressor response from the IXth nerve; subsequent destruction of the dorso-lateral part of the medulla 2 mm caudal to the obex, however, did.

These observations demonstrate that, like the TDR, depressor responses mediated by the IXth and Xth nerves, other than those arising from arterial baroreceptors, are not dependent upon the integrity of NTS. Moreover, since both the TDR and non-baroreceptor depressor responses are abolished by lesions of the dorsolateral cord including the spinal trigeminal tract in the cervical-medullary region, the spinal trigeminal system may serve as a site of integration of some depressor reflexes from receptors in the head and neck.

DISCUSSION

1. THE TRIGEMINAL DEPRESSOR RESPONSE

The present study has demonstrated that low frequency electrical stimulation within portions of the trigeminal complex of anesthetized or decerebrated rabbits will elicit a patterned autonomic response consisting of a decrease in heart rate, a fall of arterial pressure, expiratory apnea and an increase in gastric motility. For convenience, we have termed this pattern of autonomic effects the trigeminal depressor response (TDR)²⁸ in reference to its apparent site of integration within the spinal trigeminal complex.

The pattern of autonomic responses comprising the TDR has never, to our knowledge, been previously observed from electrical stimulation of the spinal trigeminal complex. Although, on occasion, electrical stimulation within the system has been reported to elicit bradycardia,^{17,47} an associated hypotension has never been seen. The failure of others to detect the TDR may be related to the fact that the spinal trigeminal complex has infrequently been explored for cardiovascular responses^{1,11}. In those few studies in which it has,^{31,51} the stimulus frequencies appear to have been well above the low frequencies required to elicit it.

Autonomic Mechanisms.

The changes in arterial pressure, heart rate, and gastric motility associated with the TDR appear to result from the inhibition of ongoing sympathetic nerve activity and/or excitation of vagal neurons. The fall of arterial pressure seems to be exclusively due to a reduction of peripheral resistance, as a consequence of inhibition of sympathetic activity, since: (a) the cardiac output is unchanged during the depressor (hypotensive) response; (b) hypotension occurs when the bradycardia is abolished by a combination of vagotomy and β -adrenergic blockade; and (c) the hypotension can be blocked by α -adrenergic blockade and not by atropine (and hence is unlikely to be due to sympathetic cholinergic vasodilator fibers⁵⁰). The bradycardia, on the other hand, is due to an immediate excitation of the cardiac vagus and a more prolonged sympathetic inhibition. Gastric hypermotility appears to be exclusively vagal.

Anatomical Distribution.

With low threshold stimulation the TDR can only be elicited from topographically restricted regions of the trigeminal complex. Thus, a full response was elicited from the area of the entering roots, through the entire extent of the spinal tract of V, particularly in its dorsal and medial portions, and in caudal portions of the nucleus of the spinal tract, particularly the subnucleus caudalis^{10,39,45}. Stimulation of the principal sensory, motor, or mesencephalic nuclei of V failed to elicit any cardiovascular responses.

The discrete localization of low threshold sites in the trigeminal complex and the disappearance of the response with focal lesions indicates that the response cannot be attributed to spread of current to structures outside of the trigeminal system. However, the fact that most of the entering rootlets of the IXth and Xth cranial nerves penetrate the spinal tract of V as they precede medially to enter the NTS^{2,7,9,10,24,25,38,42,48} raises the possibility that the TDR is due to stimulation of these entering rootlets and the response, therefore, is mediated indirectly by NTS.

This latter possibility can be discounted for a number of reasons: (a) First, while the roots of IXth and Xth nerve enter the brainstem as a band of radicals^{2,7,9,12,24,25,26,48} from approximately the rostral pole of the VIIth nucleus to the obex, a TDR of substantial amplitude was elicited from sites in the trigeminal system, both rostral and caudal to this root entry zone (Fig. 3). (b) Second, a powerful response was elicited within the zone of the entering roots of the Vth nerve, a region considerably rostral and ventral to the site of root entries of IX and X. (c) Third, while the rootlets of IX and X proceed medially in a continuous band through the spinal trigeminal system and across the brainstem into the NTS, a TDR was never elicited from sites medial to spinal V (see Fig. 3). (d) Fourth, maximum responses were elicited at any site in the spinal tract of V. This finding would be consistent with the interpretation that the response is due to stimulation of a discrete and

compact descending bundle of fibers, but difficult to explain as an effect of stimulation of only a few afferent rootlets at any electrode site. (e) Fifth, punctate electrical stimulation within the trajectory of the penetrating roots of IX and X has never been found to elicit any antidromic activity in the carotid sinus and aortic depressor nerves ¹³. Stimulation of rootlets in NTS, however, will. This fact indicates that local stimulation will, at best, only excite few fibers of IX and X. (f) Finally, lesions of NTS which blocks baroreceptor reflexes, a major vasodepressor reflex mediated by the IXth and Xth nerves, fails to abolish the TDR. This observation also rules out the possibility that fibers of the Vth nerve which leave the trigeminal nucleus to join the NTS^{44,48} mediate the TDR.

The observation that the most sensitive sites for the TDR in the spinal tract of V are dorsal is of interest. While dorsal portions of the spinal tract contain fibers from the mandibular division of the Vth nerve, it also contains those branches of the IXth and Xth nerve which leave the main bundles of penetrating rootlets to project caudally, along with trigeminal fibers, into the spinal cord^{2,9,10,12,24,25,46,48}. It is therefore probable that electrical stimulation in the spinal tract of V, will excite, in addition to fibers of the Vth nerve, descending branches of the IXth and Xth cranial nerves in the trigeminal system. This interpretation would also explain our observation that

lesions which destroy the spinal trigeminal tract at the cervical-medullary junction also abolish vasodepressor responses arising from stimulation of the main trunks of the IXth and Xth nerves in the periphery. These observations suggest that there may also be an organization of autonomic representation within the dorsal portions of spinal tract of V.

2. THE TDR AS AN AUTONOMIC REFLEX FROM THE TRIGEMINAL NERVE

That the TDR can result from stimulation of fibers of the Vth nerve has been clearly demonstrated in this study: Electrical stimulation of any branch of the trigeminal nerve or, of the entering roots, will elicit hypotension and bradycardia; the response from the nerve shares the same stimulus frequency characteristics as that elicited from the brain; and, the responses from the periphery are abolished by lesions of the spinal trigeminal complex but not by lesions of NTS. These observations indicate that the TDR can be elicited as a reflex response from stimulation of receptors innervated by branches of the trigeminal nerve.

It should be emphasized that the TDR, by virtue of the associated hypotension, differs from all other autonomic reflexes elicited by natural stimulation of trigeminal receptors, including the diving, the nasopharyngeal, and the oculocardiac responses. Both the diving reflex, initiated

in diving^{16,37} and non-diving³ vertebrates (including rabbits⁴⁹), by submersion of the face, and the nasopharyngeal reflex^{4,52} initiated by noxious stimulation of the olfactory mucosa, share with the TDR apnea and bradycardia. However, they differ from the TDR, in that they are both associated with a marked increase in peripheral resistance (often associated with hypertension) and a reduced cardiac output. The oculocardiac (Aschner) reflex, which has been infrequently studied, is not associated with a fall of arterial pressure.⁵

The only report known to us in which hypotension was elicited from the trigeminal nerve or its branches is a study by Dellow and Morgan¹⁴. They observed that electrical stimulation of the lingual nerve or tooth pulp in anesthetized cat elicited a fall of arterial pressure of up to 20 mmHg. The response was more effective at 10 Hz than 60 Hz. Although other cardiovascular and respiratory effects were not studied, it seems probable that the response they observed was a TDR.

The nature of the adequate stimulus, the identity of the receptors for the TDR and the characteristics of the afferent fibers which mediate it are unknown. Since it can be evoked from all branches of the trigeminal nerve, it is unlikely to arise from a specific cranial organ (e.g. the eye or teeth); nor can it be related to a specific division of the Vth nerve, even though within the spinal tract of V the response is elicited from dorsal portions, an area believed to contain fibers of the mandibular division.¹⁰

Also, since the TDR can be elicited from stimulation of the supra- and infraorbital nerves (which are cutaneous and thus do not innervate joints or muscles) but not by stimulation within the mesencephalic nucleus suggests that the response does not arise from proprioceptors.

The widespread distribution of receptors for the response, its similarity to the vasodepressor response elicited by group III afferents in muscle²³, the fact that it most likely can be elicited by tooth pulp stimulation¹⁴, and recent observation by us (Kumada, Dampney, Reis, unpublished) that with stimulation of nerve or spinal tract of V, the threshold lies in the range of A-delta fibers, raises the possibility that TDR may be related to pain mechanisms.

3. THE RELATIONSHIP OF THE TDR TO SOME OTHER

VASODEPRESSOR REFLEXES

(a) Relationship to Arterial Baroreceptor Reflexes

The TDR bears a striking similarity to arterial baroreceptor reflexes evoked from receptors in the carotid sinus and aortic arch. Both the TDR and baroreceptor responses are characterized by a fall of arterial pressure due to sympathetic inhibition,¹⁹ a bradycardia due to vagal excitation acting in concert with sympathetic inhibition,^{22,32} an unaltered cardiac output,³⁰ expiratory apnea¹⁸ and a vagally mediated increase in gastric motility.^{8,27} Of particular interest is the fact that the TDR, when elicited by electrical stimu-

lation of the spinal trigeminal complex, appears to be as large in magnitude as that which can be elicited by stimulation of arterial baroreceptors.

It is now well recognized that arterial baroreceptor reflexes are mediated in large measure through synapses in the nucleus tractus solitarii (NTS), particularly that portion of the nucleus which lies near the obex (the intermediate third).^{12,20,29,34} Bilateral electrolytic lesions at this site in cat,³⁵ rat¹⁵ and, as demonstrated here, in rabbit, will abolish all reflex responses from arterial baroreceptors elicited electrically or by natural stimulation.

On the other hand, lesions of NTS, while abolishing the arterial baroreceptor reflexes, fail to block the TDR. Conversely, lesions of the spinal trigeminal complex which abolish the TDR are without effect on arterial baroreceptor reflexes. These findings indicate that the TDR and arterial baroreceptors are, at least in part, separately organized within the brain. The former depends upon the integrity of the spinal trigeminal complex and perhaps its projection into the spinal cord, the latter on NTS. However, the possibility that these two responses converge at some locus, for example, at restricted areas in the reticular formation, or in spinal cord, to initiate a common pattern of autonomic activity remains a possibility.

(b) Relationship to Depressor Reflexes mediated by the IXth and Xth Cranial Nerves Other Than From Arterial Baroreceptors

Bilateral lesions of the spinal trigeminal complex, but not of NTS, also abolished the hypotension and bradycardia reflexly elicited by electrical stimulation of the proximal ends of the IXth and Xth cranial nerves (after exclusion of the carotid sinus and aortic depressor nerves). These findings raise three points: First, they suggest that the TDR can also be initiated reflexly by excitation of afferent fibers of the IXth and Xth cranial nerves innervating receptors distinct from arterial baroreceptors. Second, that the afferent fibers mediating the response descend in the spinal trigeminal system and probably consist of those anatomically well-defined branches of the IXth and Xth nerve which leave the entering bundle of roots as the latter penetrates the spinal tract of V and descend with trigeminal fibers to terminate largely in the upper cervical cord^{2,9,10,24,25,48}. Third, that some of the vasomotor responses elicited by electrical stimulation in spinal tract at V are probably due to stimulation of descending fibers from IXth and Xth nerve as well as fibers from the Vth cranial nerve.

The nature of the receptors and their adequate stimuli is obscure. Possible candidates would be the pharyngeal, pulmonary, cardiac and conceivably other abdominal visceral

receptors innervated by the IXth and Xth nerves and from which vasodepressor responses have been reported.⁴¹ These might, for example, include the reflex originating from J receptors of the lung,⁴⁰ stretch reflexes from the left atrium and ventricle,⁶ and possibly, depressor responses from abdominal receptors.²¹

(c) Relationship to Depressor Responses from Spinal Afferent Nerves

It has long been known that bradycardia, apnea and a fall of arterial pressure, can be reflexly elicited by low frequency electrical stimulation of spinal nerves such as the sciatic. (e.g. 23,43) This response has been termed the somatic depressor response. Detailed studies by Johansson²³ have shown that the responses from spinal nerves are primarily due to activation of receptors in muscle innervated by group III afferent fibers. High threshold cutaneous receptors may also yield a small depressor response. The anatomical evidence would suggest²³ that the somatic depressor response is mediated by an ascending system passing through the anterolateral fasciculus of the spinal cord, possibly in association with spinothalamic tracts. This pathway passes through the ventromedial portions of the lower brainstem. Lesions of the anterolateral fasciculus of the cord or of the ventromedial portions of the brainstem will abolish all the pressor responses arising from spinal nerves²³. It should

be emphasized that at this level of the lower brainstem, the TDR is only abolished by lesions damaging the spinal tract of V. These facts suggest, therefore, that in part the central neuroanatomical organization of the TDR and the somatic depressor responses differ. However, it still is possible that these two vasodepressor systems may share common sites of integration outside of the trigeminal and spinothalamic systems, possibly residing in the reticular formation or even in spinal cord.

4. SUMMARY

The TDR appears to represent a new and powerful vasodepressor reflex organized within the spinal trigeminal system of the brain. It is reflexly initiated from as yet unidentified receptors in the trigeminal nerve and from visceral receptors, other than arterial baroreceptors, innervated by branches of the IXth and Xth cranial nerves. These probably represent those fibers of IX and X which enter the spinal tract of V. The TDR is distinct on the basis of its dependence on the integrity of the spinal trigeminal complex, from the depressor responses from arterial baroreceptors innervated by the carotid sinus and aortic depressor nerves, and from the vasodepressor responses elicited from stimulation of spinal nerves, particularly from muscle.

The function of the TDR remains obscure and many questions remain to be answered. For example, does it function

tonically to inhibit sympathetic tone as do baroreceptors?
Which specific physiological conditions activate the TDR?
Does the TDR affect such neuronal functions as conscious or
emotional states which involve brain structures higher than
the trigeminal system? An interesting finding in this
connection is that the trigeminal system appears to partici-
pate in autonomic and behavioral functions such as feeding
and drinking, which are independent of the role of the
system in somatosensory perception.⁵⁴ Our findings that the
spinal trigeminal complex modulates arterial pressure and
heart rate add a new facet to the evidence that the tri-
geminal system may serve to link the somatic and autonomic
nervous system.

ACKNOWLEDGMENTS

Supported by National Institutes of Health Research Grants (HL 16834, NS 3346, HL 18974) and a Grant (NGR 33-010-179) from the National Aeronautical and Space Administration. Roger A.L. Dampney is an Overseas Research Fellow of the Life Insurance Medical Research Fund of Australia and New Zealand.

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FIGURE LEGENDS

Fig. 1. Elicitation of the trigeminal depressor response in brainstem of anesthetized rabbit. Upper drawing represents a coronal section of brainstem at 6 mm anterior to obex from a representative experiment. Four electrode tracts are shown with stimulus sites 500 μ m apart, illustrated by filled and open circles. At each site the brain was stimulated with a 12 sec stimulus train (0.5 msec duration, 5 Hz and constant intensity of 100 μ A). Positive responses are identified by filled, negative by open circles. The most lateral track with letters a-e corresponds to polygraph tracings in lower tracing. Note that the trigeminal depressor response is only elicited from sites within the spinal trigeminal complex. See Fig. 3 for abbreviations.

Fig. 2. Changes in threshold current along an electrode tract required to elicit the trigeminal depressor response in anesthetized rabbit. Coronal section on the left corresponds to a section of brain 5 mm anterior to the obex with an electrode track passing through the spinal trigeminal tract and its nucleus. The brainstem was stimulated with 12 sec trains of square wave pulse (0.5 msec pulse duration, 5 Hz) at 250 μ m steps and the threshold current determined. Graph on right represents threshold current required to elicit a threshold response (i.e., the minimum current that

elicited a depressor response greater than 10 mmHg).

Filled circles represent brain sites at which the threshold current was smaller than or equal to 200 μ A. The ordinate on the right represents the depth from the surface in mm; the abscissa represents stimulus intensity in microamperes. See Fig. 3 for abbreviations. Note that the sites with lowest threshold are within the spinal trigeminal tract.

Fig. 3. Localization in the spinal trigeminal complex of rabbit of sites from which a depressor response is elicited by electrical stimulation. Coronal sections of brainstem at 12-14 mm anterior (a), 10-11 mm anterior (b), 8-9 mm anterior (c), 6-7 mm anterior (d), 4-5 mm anterior (e), 2-3 mm anterior (f), 0-1 mm anterior (g), 1-2 mm posterior (h), and 3-4 mm posterior to the obex. The brainstem was briefly stimulated every 250 μ m with a 12 sec train of square wave pulse (0.5 msec pulse duration, 5 Hz) and the threshold current was determined at each site as in Fig. 2. Each line of circles represents an electrode track. Thresholds for responses are represented by large and small filled circles and small open circles. Data were obtained from 22 anesthetized rabbits. Abbreviations: V, trigeminal nerve; VII, facial nerve; VIII, stato-acoustic nerve; Ant horn, anterior horn; Brc, superior cerebellar peduncle (brachium conjunctivum); Brp, middle cerebellar peduncle (brachium pontis); Cgs, central gray substance; Cn+Gn, nucleus cuneatus and nucleus gracilis; Coch, cochlear nucleus; Cr, inferior cerebellar peduncle (restiform body); Dvn, inferior vestibular nucleus; Lc,

locus coeruleus; Lvn, lateral vestibular nucleus; Mvn, medial vestibular nucleus; NVmt, motor nucleus of trigeminal nerve; N VII, nucleus of facial nerve; N XII, nucleus of hypoglossal nerve; N caud V, nucleus caudalis of trigeminal nerve; N ip V, nucleus interpolaris of trigeminal nerve; NmX, dorsal motor nucleus of vagus; NoV, nucleus oralis of trigeminal nerve; N pr V, nucleus principalis of trigeminal nerve; Nts, nucleus of solitary tract; Oli, inferior olive; Ols, superior olive; Ph, nucleus praepositus hypoglossi; Pyr, pyramidal tract; Svn, superior vestibular nucleus; Tr sp V, spinal tract of trigeminal nerve. Note that the sites with lowest threshold are within the spinal trigeminal complex.

Fig. 4. Bradycardia and hypotension elicited by electrical stimulation of branches of trigeminal nerve. Supraorbital (a), infraorbital (b), inferior alveolar (c) or lingual nerve of anesthetized rabbits was stimulated with a 12 sec stimulus train (0.5 msec duration, 5 Hz) at intensities of 0.3 mA, 1 mA, 1 mA or 0.5 mA, respectively, as indicated by the upward deflection of the bottom tracing of each record. The data were obtained from 3 experiments.

Fig. 5. a: Stimulus frequency-response characteristics for the hypotension elicited by electrical stimulation of the spinal trigeminal complex (broken line) or branches of the Vth nerve (solid line). Stimulus consists of a 12 sec

train of square wave pulse (0.5 msec pulse duration, 5 Hz) of 5 times threshold intensity. The response is expressed as change in arterial pressure (in mmHg) from the prestimulus level. Data were obtained from 6 different experiments. Note that optimal stimulus frequencies are same for brain and nerve, lying between 5-20 Hz and that with stimulus frequencies at 50 Hz or higher no response or a pressor response is seen. b: Stimulus intensity-response characteristics for the hypotension elicited by electrical stimulation of branches of the Vth nerve (solid line) or the spinal trigeminal complex (broken line) from 6 representative experiments. Stimulus was a 12 sec train of square wave pulse (0.5 msec duration, 5 Hz) over a range of 1 to 10 times threshold current. Note that the maximum response is obtained at stimulus intensities of 3 to 6 times threshold.

Fig. 6. Effect of vagal and cardiac sympathetic blockade, alone or combined, on trigeminal depressor response. The nucleus caudalis was electrically stimulated in anesthetized paralyzed rabbits (0.5 msec pulse duration, 5 Hz, 12 sec train duration at 100 μ a). a: Control; b: 5 min after vagotomy; c: 10 min following administration of propranolol (1 mg/kg i.v.) (vagi sectioned); d: Control in another experiment; e: 10 min following administration of propranolol (1 mg/kg i.v.) (vagi intact). Note that both vagal and sympathetic blockade are required to abolish bradycardia while hypotension is unaffected.

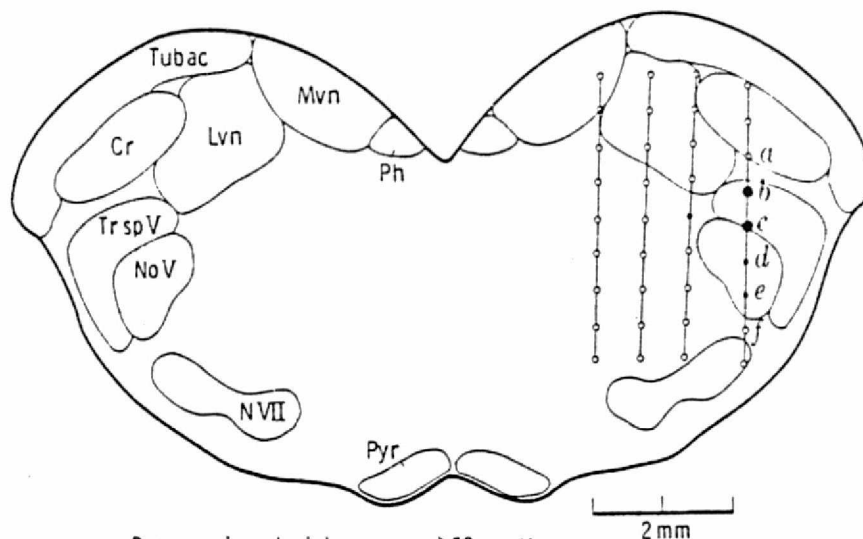
Fig. 7. Effect of α and β sympathetic adrenergic blockade, alone or combined, on bradycardia, hypotension and cardiac output elicited by stimulation of the trigeminal nucleus. The nucleus caudalis was electrically stimulated in an anesthetized paralyzed rabbit (0.5 msec pulse duration, 5 Hz, 12 sec train duration at 100 μ A). a: Control; b: 10 min following administration of propranolol (1 mg/kg i.v.); c: Phentolamine administered 10 min after propranolol (1 mg/kg i.v.); d: Control in another experiment; e: 10 min after administration of phentolamine (1 mg/kg i.v.). Note that phentolamine blocks most of hypotension and that cardiac output is unchanged during the trigeminal depressor response.

Fig. 8. (a) Changes in respiration during the trigeminal depressor response in a spontaneously breathing anesthetized rabbit elicited by electrical stimulation of the spinal trigeminal tract at the level of the VIIth nucleus. The stimulus was a 12 sec train (0.5 msec pulse duration, 5 Hz, 200 μ A). With slightly lower stimulus intensities the response consisted of tachypnea. Note apnea in expiration. (b) Changes in intragastric pressure during electrical stimulation of the nucleus caudalis in a paralyzed anesthetized rabbit. Increased contractility was only partially abolished by ipsilateral or contralateral vagotomy. Bilateral vagotomy abolished the response.

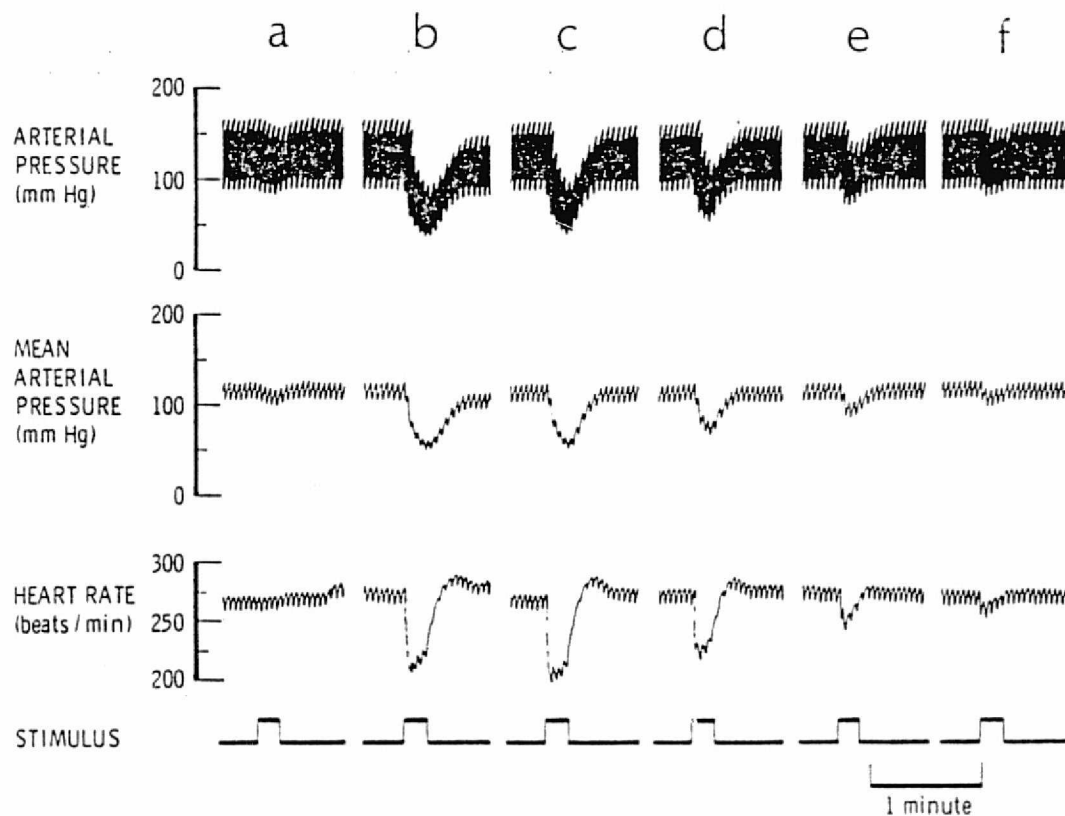
Fig. 9. Persistence of trigeminal depressor response after abolition of arterial baroreceptor reflex by bilateral lesions in nucleus tractus solitarii (NTS). a and b: Before NTS lesions. a: A rise of arterial pressure accompanied by a reflex bradycardia of the arterial baroreceptor origin caused by intravenous injection of norepinephrine ($2 \mu\text{g/kg}$); b: The trigeminal depressor response elicited by electrical stimulation of the nucleus caudalis (0.5 msec pulse duration, 5 Hz, 12 sec train duration at $100 \mu\text{A}$). c and d: Following bilateral electrolytic lesions of NTS near the obex. c: Elimination of the baroreceptor reflex caused by norepinephrine injection by NTS lesion; d: Persistence of the trigeminal depressor response elicited by stimulation of the nucleus caudalis after NTS lesions.

Fig. 10. Effects of lesions of the spinal trigeminal complex and the nucleus tractus solitarii (NTS) on the depressor response elicited from the Xth and aortic depressor (AN) nerves. a and b: Control. a: Hypotension with bradycardia elicited by electrical stimulation of the left aortic nerve (AN) (0.5 msec pulse duration, 5 Hz, 12 sec train duration at $50 \mu\text{A}$); b: Hypotension with bradycardia elicited by electrical stimulation of the remainder of the cervical vagus (Xth) nerve distal to the junction of the aortic nerve (0.5 msec pulse duration, 5 Hz, 12 sec train duration at $200 \mu\text{A}$). c and d: After destruction of most of the spinal trigeminal complex on the left side of the brainstem (2 mm

posterior to the obex). The destruction abolishes the response to stimulation of the Xth nerve (d), but not the depressor reflex from the aortic nerve (c). e and f: Another experiment. Effects of electrolytic lesions in the NTS. The NTS lesion eliminates the depressor reflex elicited by stimulation of the ipsilateral aortic nerve (e), but not the depressor response from the Xth nerve (f).

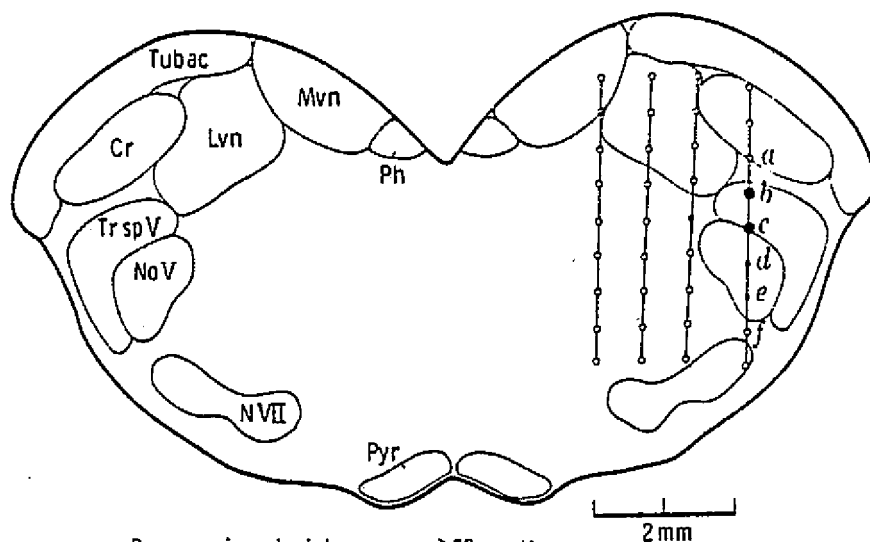


- Decrease in arterial pressure >50 mm Hg
- Decrease in arterial pressure 10-50 mm Hg
- Decrease in arterial pressure ≤ 10 mm Hg

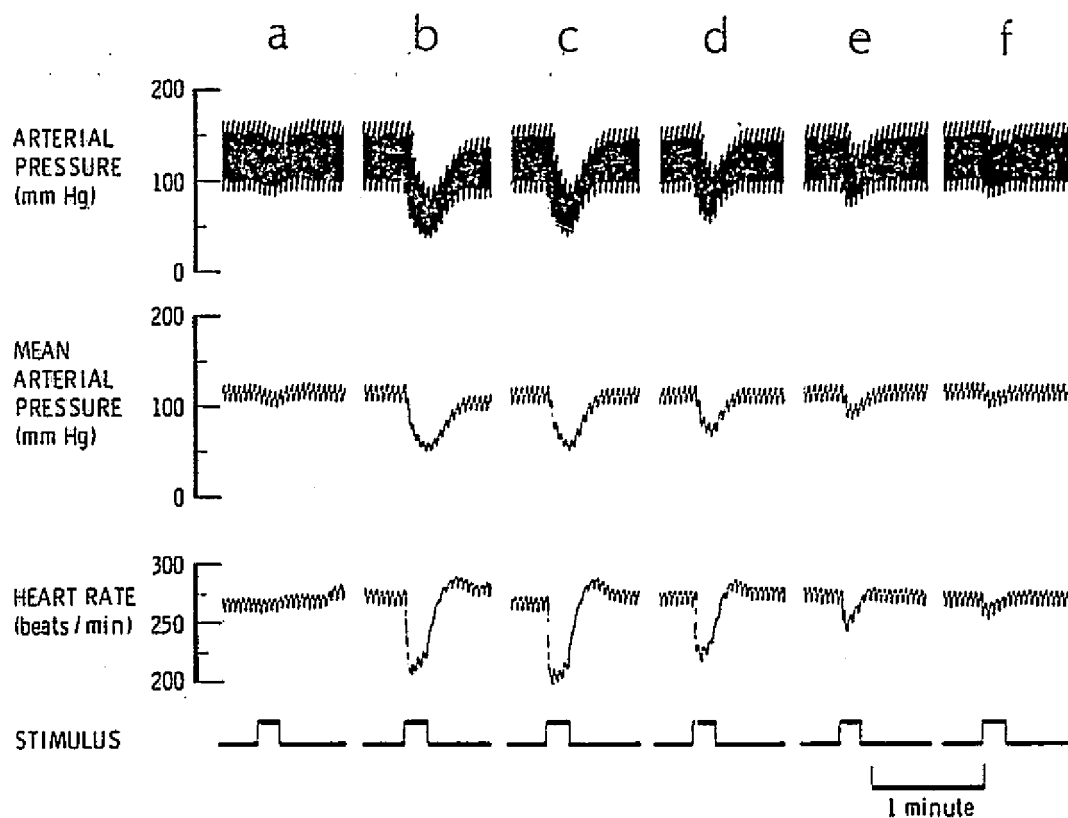


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Figure 1.



- Decrease in arterial pressure >50 mm Hg
- Decrease in arterial pressure 10-50 mm Hg
- Decrease in arterial pressure ≤ 10 mm Hg



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Figure 1.

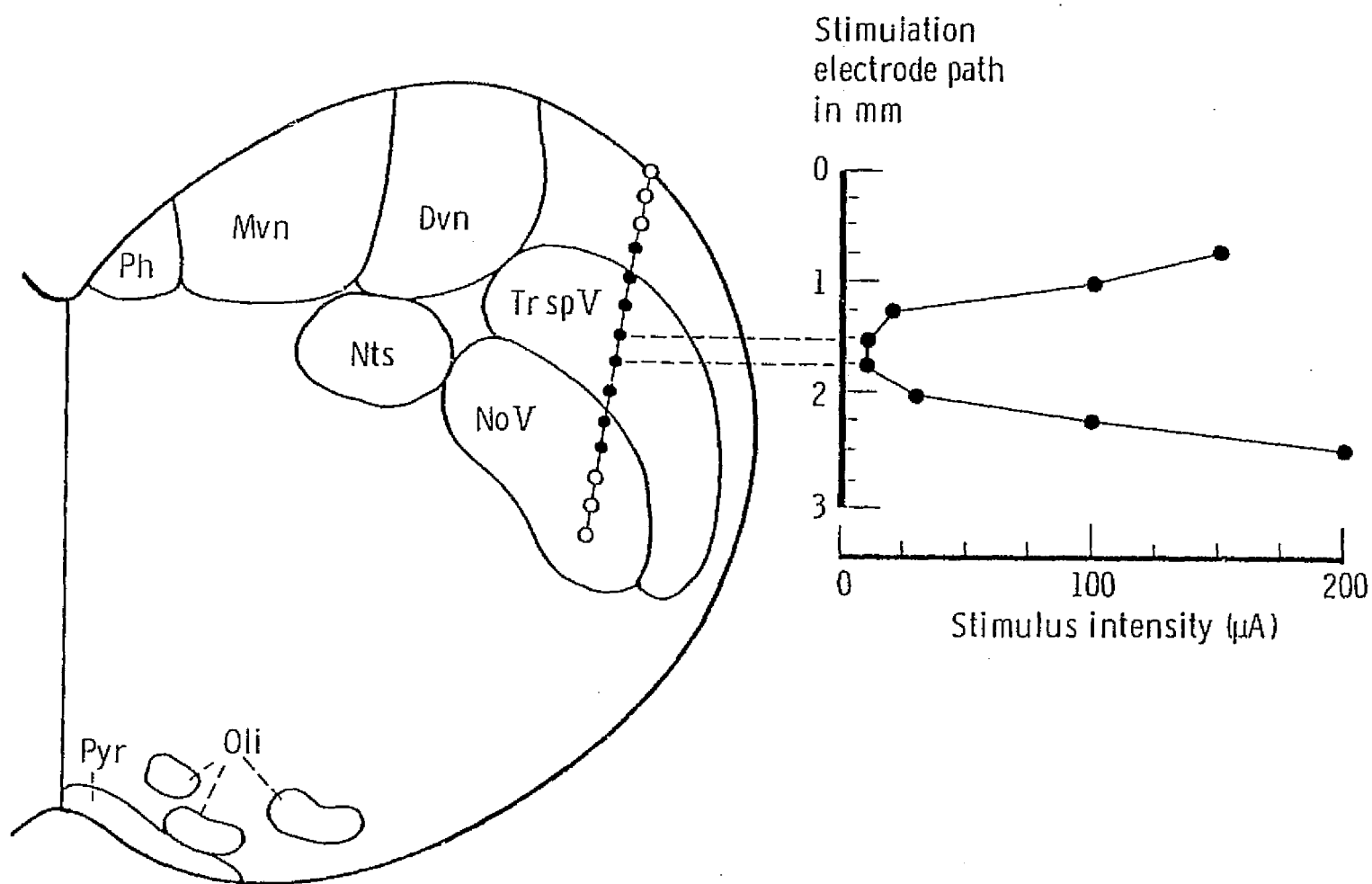
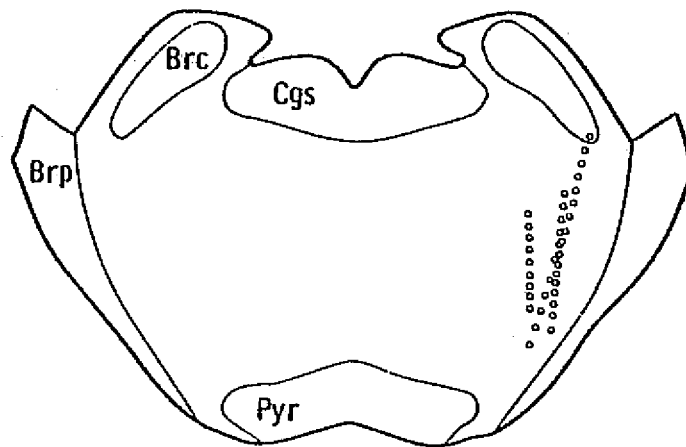
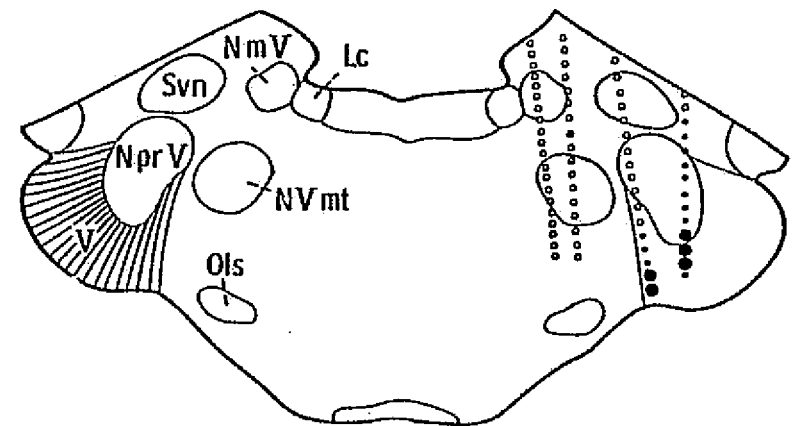


Figure 2.

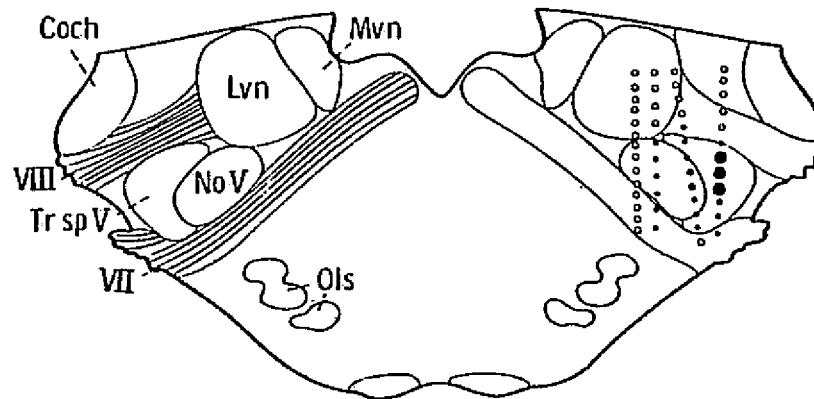
a A 12-14



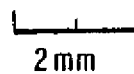
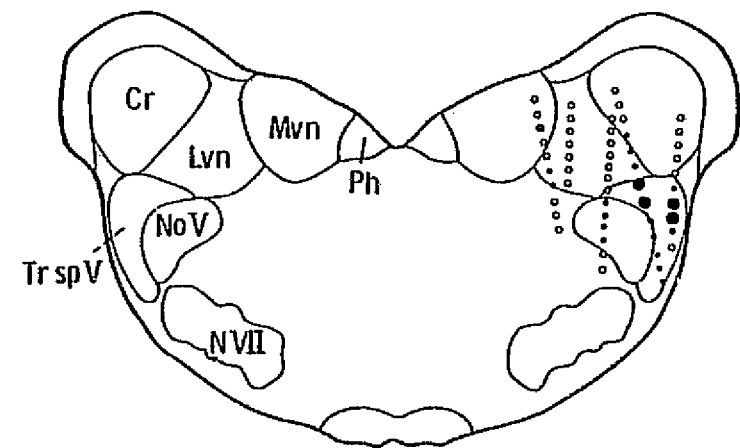
b A 10-11



c A 8-9



d A 6-7



- Threshold current $\leq 10\mu\text{A}$
- Threshold current 10 - $100\mu\text{A}$
- Threshold current $>100\mu\text{A}$ or no response

Figure 3. Part 1.

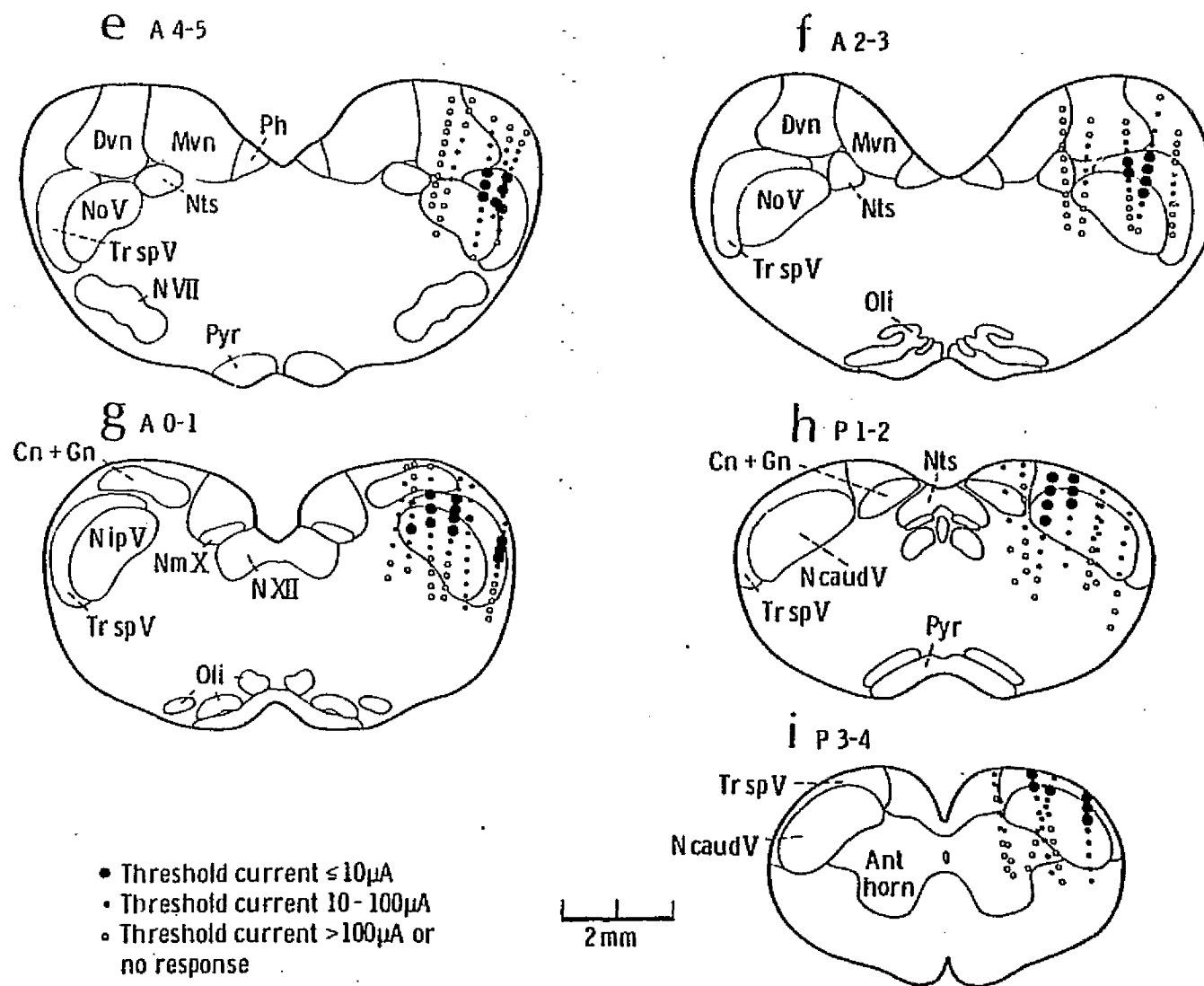


Figure 3. Part 2.

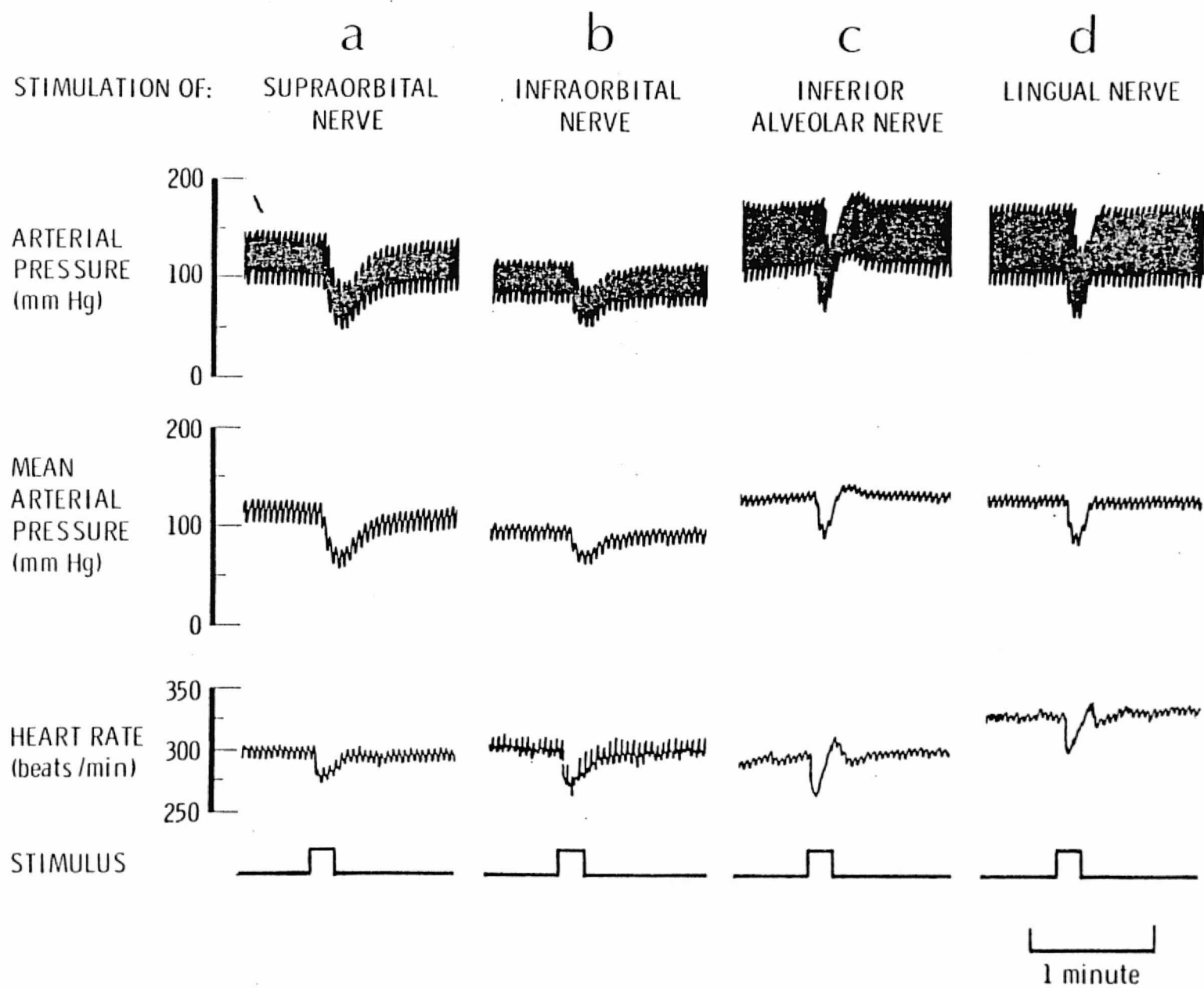


Figure 4.

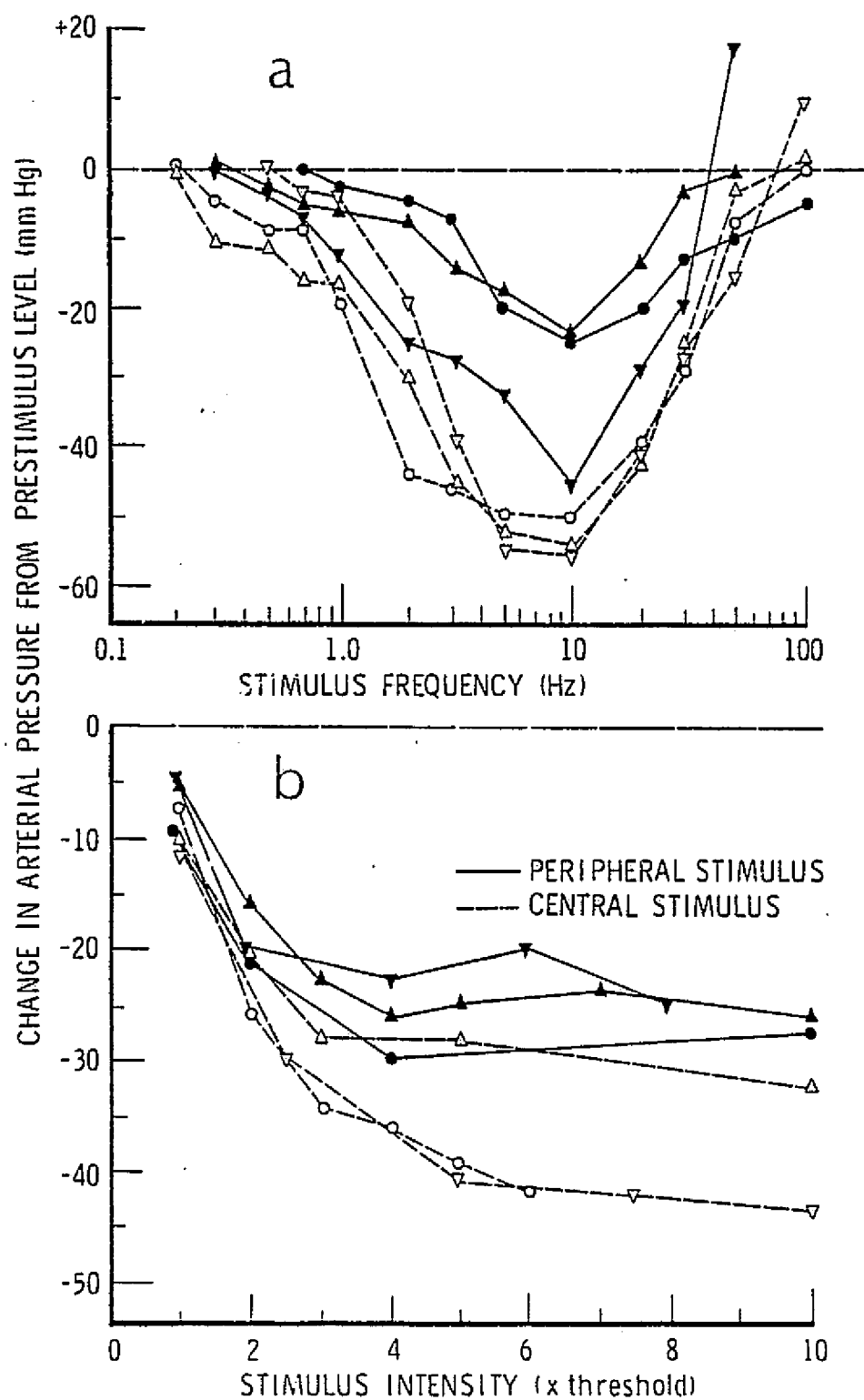


Figure 5.

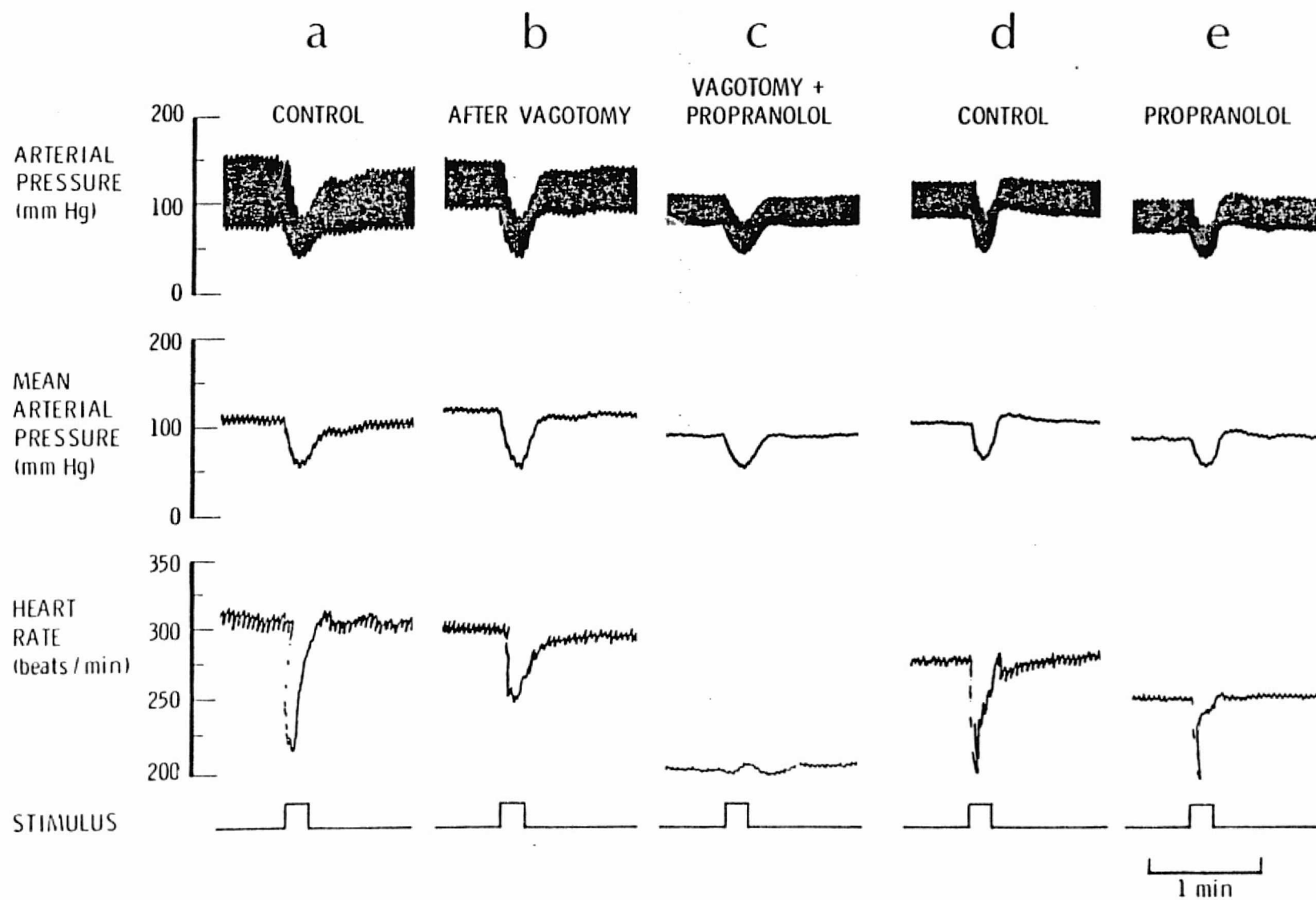


Figure 5.

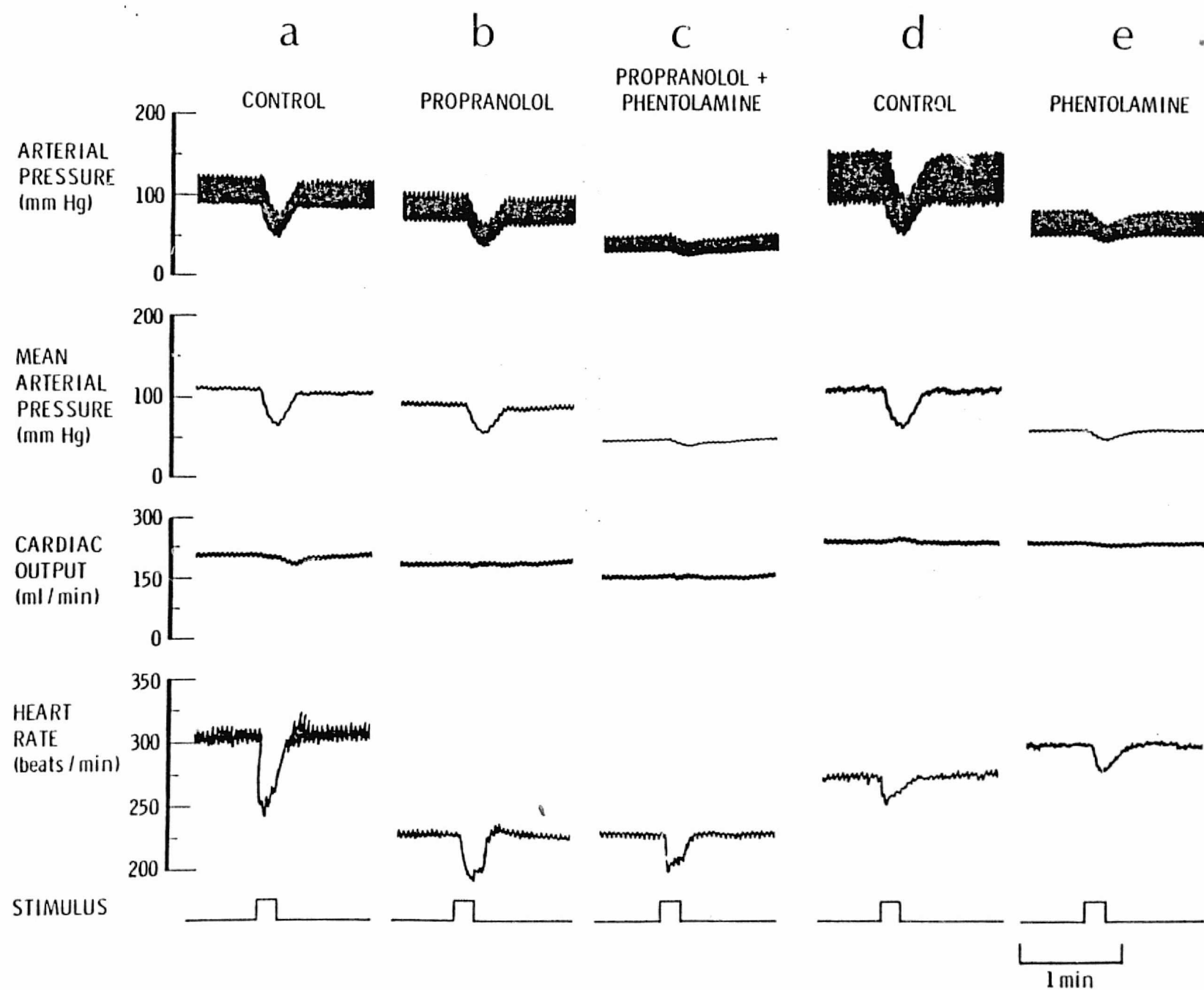


Figure 7.

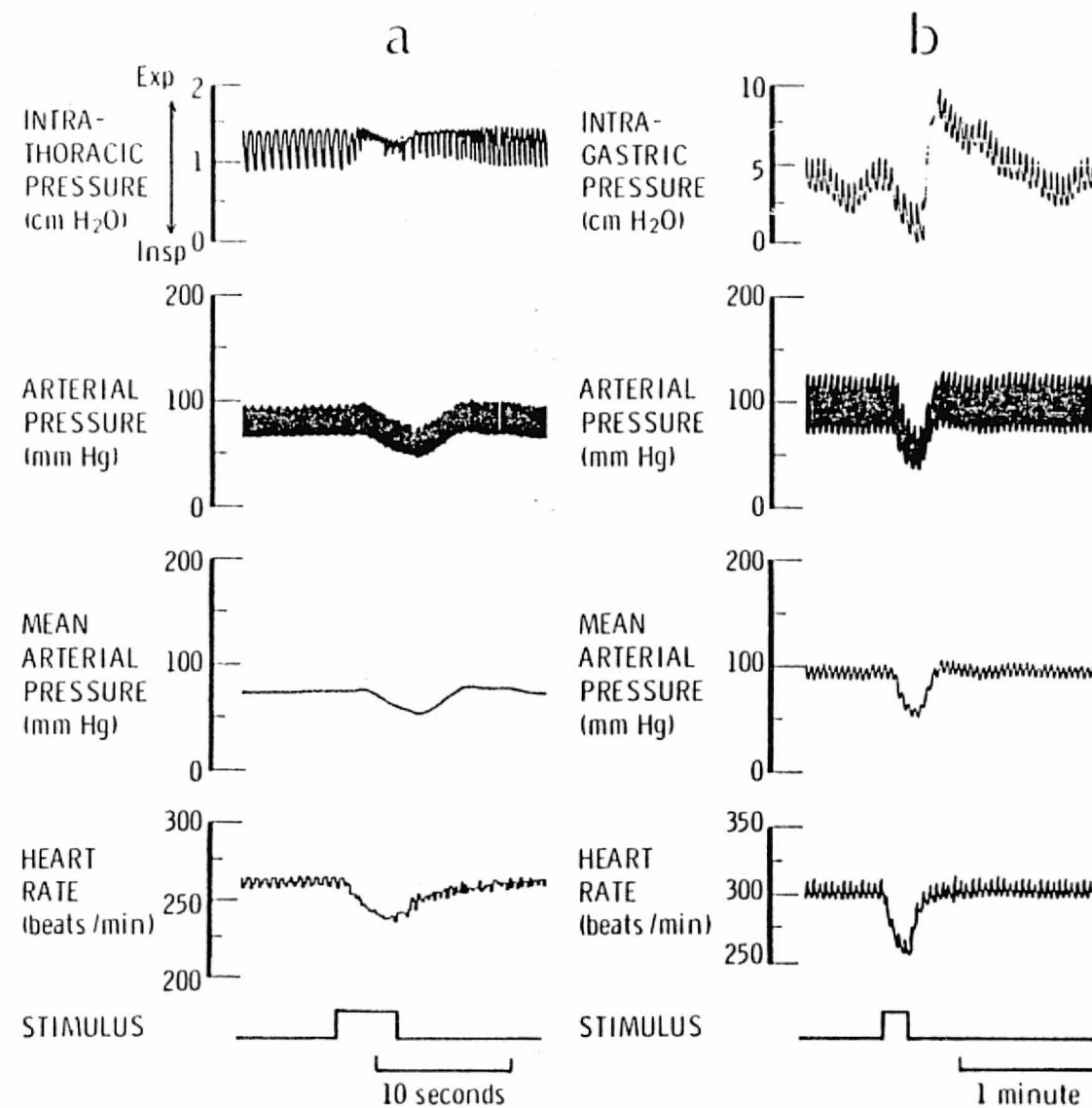


Figure 8.

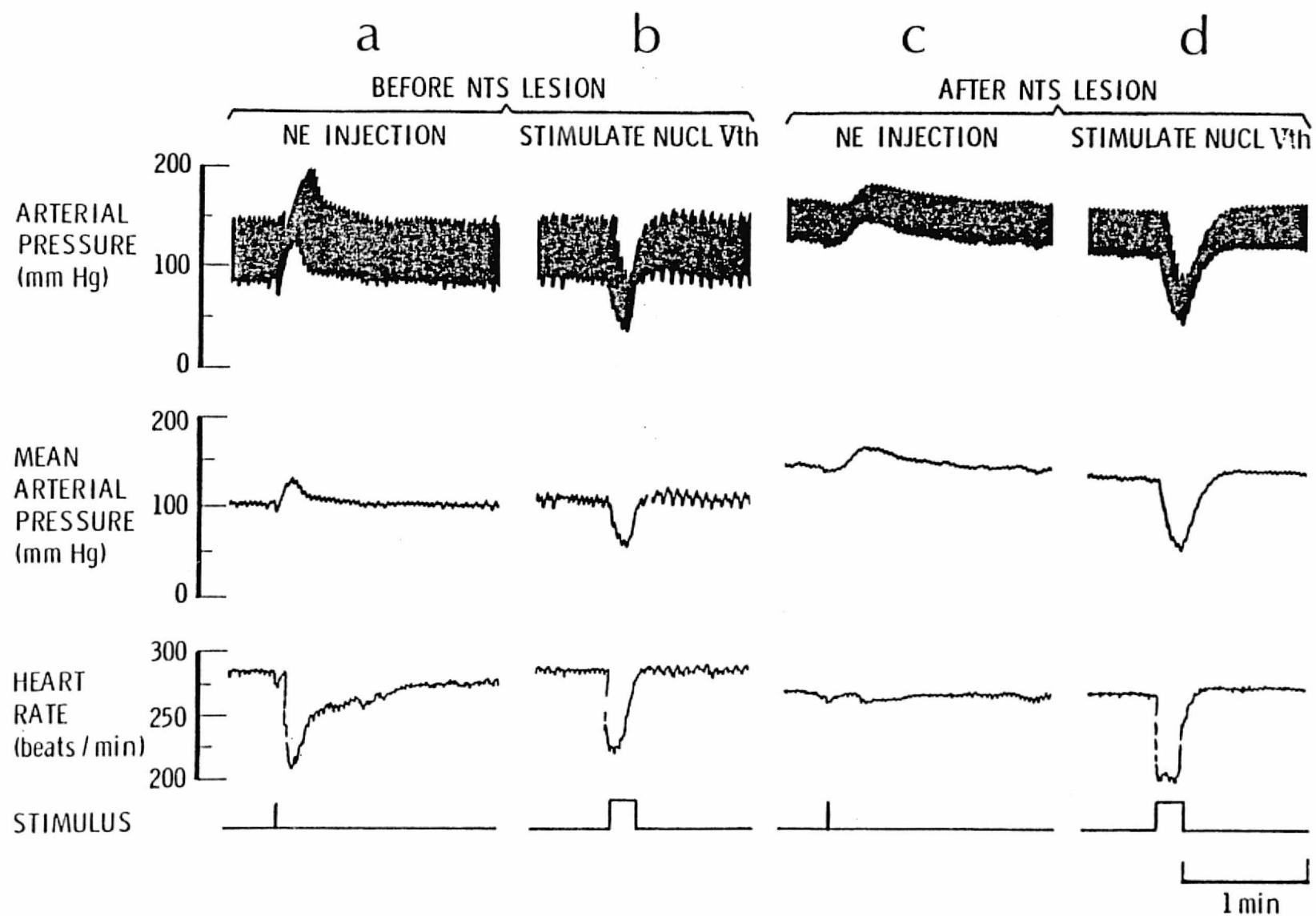


Figure 9.

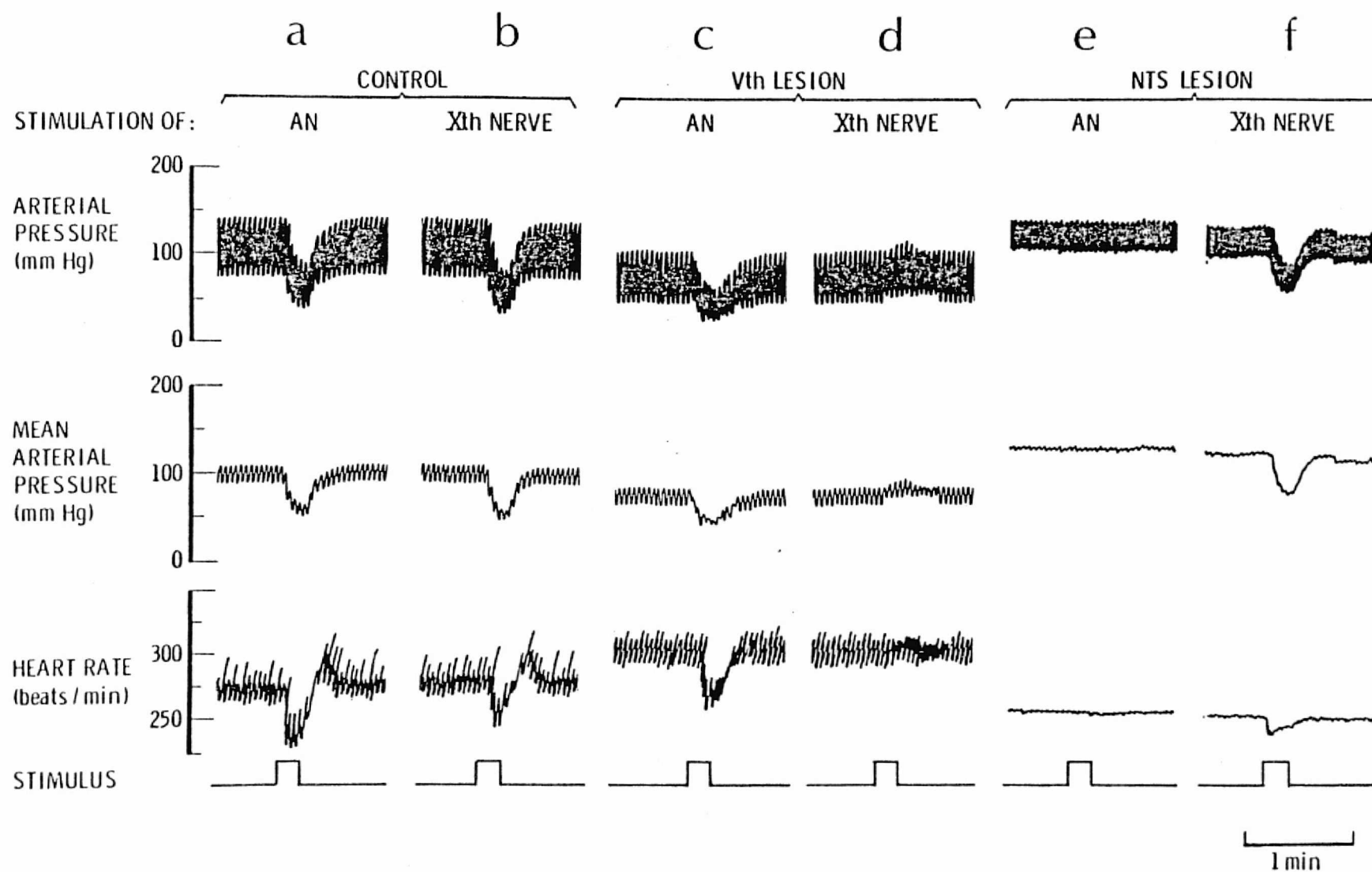


Figure 10.

CHRONIC LABILE HYPERTENSION PRODUCED IN CAT.
BY LESIONS OF THE NUCLEUS TRACTUS SOLITARI

by

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and

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This work was supported by Research Grants
HL 18195 and HL 18974 from NHLI, Grant NS 03346
from NINDS and NGR-33-010-179 from NASA.

M.A.N. is a recipient of a Research Career Development
Award from NHLI.

Abbreviated Title: Hypertension in Cat from Brain Lesions.

ABSTRACT

Bilateral electrolytic lesions of the nucleus tractus solitarii (NTS) were made at the level of the obex in 7 cats. Within 1 hour after the lesions the mean arterial pressure (MAP) rose to a maximum of 144 mmHg (141% of control), and by 7 hours heart rate reached a high of 236 bpm (148% of control). The baroreceptor reflexes were abolished. After 24 hours the arterial pressure became extremely labile with variations of 80-100 mmHg observed. The lability occurred spontaneously and during behaviors that were self-initiated or elicited by environmental stimuli. The MAP in the lesion group was 144 mmHg (180% of control) during the day, and 96 mmHg (120% of control) at night. The lability, measured by the standard deviation, during the day in the lesion group was four times greater than in the control group and at night there were no differences. The heart rate of the lesion group was always higher than that of the control group but the lability of both groups was the same. We conclude that lesions of the NTS produced labile hypertension, probably by disinhibition of sympathetic activity through central interruption of the baroreceptor reflexes. The higher, more labile arterial pressures during the day may be caused by uninhibited increases in sympathetic activity elicited by environmental stimuli that are present during the day and absent at night. The daily variation of pressure may also be caused by somatomotor activity or by a daily rhythm of sympathetic activity which is unmasked by the lesions.

The central nervous system may play a critical role in the initiation of and/or maintenance of several models of experimental hypertension in animals (1) and possibly of essential hypertension in man (2,3). Neurogenic hypertension may result from an imbalance between systems in the brain which excite or inhibit sympathetic discharge. The imbalance could favor increased sympathetic discharge which would enhance vasoconstriction and consequently elevate the arterial pressure.

Many attempts to produce animal models of experimental hypertension have aimed at increasing sympathetic discharge either by chronic electrical stimulation of the hypothalamus, by producing brain ischemia, by subjecting animals to stress, or by behavioral conditioning (4,5). Hypertension results from these procedures but it lasts, at most, for only a few weeks.

Other studies have attempted to produce experimental hypertension by withdrawing inhibition onto sympathetic neurons by denervating the baroreceptors peripherally through transection of the carotid sinus and aortic depressor nerves (sinoaortic denervation)(1, 6-8). Most investigators are agreed that the procedure produces an increase in the lability or variation of the arterial pressure. However, there has been disagreement about the effect on the mean level of the arterial pressure. The most recent report showed that

sinoaortic denervation raised the mean level of the arterial pressure only slightly but, in agreement with earlier reports, the lability of arterial pressure was greatly increased (8). The increased lability was observed for as long as 1 year after the denervation in this study.

Recently, our laboratory used a new strategy for producing a model of neurogenic hypertension (9). The baroreceptor reflexes were interrupted centrally by bilateral placement of electrolytic lesions within the region of the nucleus tractus solitarii (NTS) in rats. This brainstem area contains the terminus of many of the afferent fibers of the baroreceptors (1,4). Hypertension rapidly developed after placement of the lesions, but it was followed within hours by cardiac failure, pulmonary edema, and death. Thus, while the procedure was successful in producing neurogenic hypertension, the hypertension spanned only a few hours.

The present study was designed to determine if NTS lesions placed in the cat would abolish the baroreceptor reflexes and produce neurogenic hypertension that would last for a longer period of time than it did in the rat. We report that NTS lesions in the cat produces an elevation in the mean level of arterial pressure and an increase in the lability of the pressure. The arterial pressure was measured for as long as 5 months after placement of the lesions and these effects were continuously present throughout the observation period.

METHODS

Animals and General Procedures

The experiments were performed on 12 vaccinated, adult cats of both sexes that were instrumented for the recording of cardiovascular activity. Lesions of the NTS were made in 7 cats. In 3 of these cats the instrumentation procedure was performed in a first operation, and 1 to 2 weeks later NTS lesions were placed in a second operation. In 4 cats, the NTS lesions and the instrumentation procedure were done at the same time. Sham lesions were made in 5 cats. In these cats the instrumentation procedure and sham lesions were performed in a single operation. All cats were observed for 1 week to 5 months following the last operation.

One to 2 days prior to surgery, a prophylactic antibiotic (sterile penicillin G benzathine suspension, 2-300,000 units, im) was administered. After induction with ether and tracheal intubation, the animals were maintained with halothane (1-2% in 50% oxygen and 50% nitrogen) delivered through a clinical anesthesia machine. All surgical procedures were performed under aseptic conditions. During surgery, body temperature was maintained at 37° (\pm 0.5° C) by a rectal probe connected to a thermostatically regulated electric heating pad. The cats were maintained on saline (0.9%, iv) throughout the surgery. All cats, after placement of the NTS

lesions or sham lesions failed to eat or drink, and spontaneous movements were reduced for about 1 week. The cats were therefore maintained on 5% dextrose and water (iv) until they were eating and drinking normally. In other respects the cats were in good health.

Implantation of Cannulas and Electrodes

The right common carotid artery and external jugular vein were exposed by splitting the sternocleidomastoid and retracting the diaphragmatic muscle. A polyvinylchloride (PVC) cannula (0.049 inches, i.d.) filled with saline containing heparin (50 units/cc) was inserted into the common carotid artery and passed into the thoracic aorta. The venous cannula was threaded down through the jugular vein to rest in the right atrium. After fixation to soft tissues, the cannulas were clamped and the free ends were threaded subcutaneously to be brought out through the skin overlying the back of the head. The arterial cannula was threaded through a 13-gauge stainless steel tube cut to a length of 1.5 cm. The tube was oriented vertically to the top of the head and cemented with dental acrylic (Kerr Manufacturing Co.) to the top of the skull. The position of all cannula tips was verified post mortem.

In some cats electrodes were implanted during the instrumentation procedure for recording extraocular movements (EOM), the electroencephalogram (EEG), and the electromyogram

(EMG) of neck muscles. The EOM and EEG electrodes were prepared in advance by soldering lengths of Teflon-insulated stainless steel wire (diameter 0.006 inches) to #0 stainless steel machine screws (80 threads/inch, 0.25 inches long). The EOM electrodes were implanted in the superior and inferior orbital ridges and wires were threaded subcutaneously to the top of the head and brought out through the skin. A midline incision was made in the skin of the head and the skull was bared for implantation of the EEG electrodes which were inserted into the skull 1-1.5 cm to either side of the midline overlying the parietal cortex. The EMG electrodes were fashioned from stainless steel wires (diameter 0.006 inches) insulated with Teflon to within 1.5 cm of the tips. The tips of the electrodes were bent into the shape of hooks. The electrodes were implanted through small skin incisions made over the paravertebral cervical musculature to either side of the midline. Movement artifact was minimized by pulling the electrodes through the muscle until the hooks were firmly anchored in the tissue. The insulated portions of the wires were then passed subcutaneously up the back of the neck and brought out through the head incision next to the other wires. All wires from the electrodes were soldered to a miniature, multipin socket (Augat, part #8058-1G68) (10). The socket was connected to the skull with the dental acrylic.

The arterial cannula was threaded through a flexible spring and one end of the spring was fitted over the metal

tube that had been cemented to the skull. After the cat was placed in the cage, the other end of the spring and the cannula were attached to a hydraulic swivel (Model #193-03, BRS/LVE, Beltsville, MD) which was mounted on top of the cage. The spring served to prevent the cannula from kinking during movement of the animal. The other end of the swivel was connected by a tube to a strain gauge transducer (Stathem, P23Db). The venous cannula was taped to the side of the spring and was connected directly to another transducer (Stathem, P23Gb) when venous pressure was recorded. At other times, the end of the venous cannula was sealed and allowed to rotate freely as the animal moved about the cage. Arterial and venous pressures were displayed on a Beckman polygraph (type RM). The peak of the arterial pressure pulse was used to trigger a cardiometer (Beckman 9857) and the heart rate was simultaneously displayed. Mean arterial blood pressure (MAP) was computed by an electronic averaging circuit (time constant = 0.53 seconds). Corrections were made to compensate for the difference in the heights of the recording transducers and the level of the heart. The cannulas were kept open by periodic flushing with heparinized saline.

Placement of NTS Lesions

The cats were placed in a stereotaxic frame with the head flexed to 45°. The atlanto-occipital membrane was visualized by separation of the posterior muscles of the

neck in the midline. The dura overlying the foramen magna was incised and the region of the obex was exposed. In most of the cats, a small portion of the posterior vermis of the cerebellum was removed by gentle suction in order to visualize the floor of the IVth ventricle.

The electrodes used for placement of the lesions in the NTS consisted of a Teflon coated stainless steel wire (diameter 0.006 inches) insulated to within 0.4 mm of the tip and carried in a 28 gauge stainless steel hypodermic tubing. Bilateral lesions were placed in the NTS at levels 0.5 mm caudal and 0.5 mm rostral to the obex; along the medial side of the posterointermediate sulcus and at a depth of 1.0-1.5 mm beneath the floor of the IVth ventricle. The lesions were made by passing a dc anodal current of 5 ma from a constant current source for 15-30 seconds. The cathode was a clip attached to the adjacent neck muscle. The control cats were operated in the same way as the cats with NTS lesions. The floor of the IVth ventricle was exposed and the posterior vermis of the cerebellum was aspirated. In one control, electrodes were inserted into the NTS but the current was not passed. The operation was completed by closure of the neck incision and discontinuation of the halothane. The cats were returned to their cages and recording of cardiovascular activity begun.

Environmental Conditions

The cats were housed in cages (67.5 cm wide x 60 cm high x 55 cm deep) that were constructed of wooden walls, a

wire-mesh floor and a door made of clear plexiglass. The cages were placed on benches in a large busy laboratory. No special attempts were made to shield the cats from ambient visual or auditory stimuli.

Construction of Frequency Histograms

Lability of arterial blood pressure and heart rate was assessed by use of frequency distribution curves that showed the number of times that a variable assumed a certain value during a selected time period. The curves were constructed by visually determining from the polygraph records the average systolic, diastolic, MAP and the heart rate during every minute over a selected one hour period. The entire range of arterial pressures and heart rates was then divided into intervals of 5mm Hg or 5 bpm and the frequencies at which the various values of arterial pressures or heart rates fell within each interval were tabulated. The reciprocals of the frequencies times 100 were plotted as percentages on the graphs of the frequency histograms.

Statistical Evaluation

Means and standard deviations were used to summarize the frequency distributions from each cat. The means describe the average of the MAP and heart rate, and the standard deviations describe the lability of these variables within

each distribution. Group statistics were computed by averaging the means, and the standard deviations from all the cats belonging to the same group. The significance of changes in the cardiovascular responses resulting from brain lesions was determined by two-tailed t-tests (11). Changes were considered to be significant at $p < 0.05$.

Behavioral Observations

In order to determine the effects of NTS lesions on the changes in arterial blood pressure and heart rate during different behaviors, the cats were observed while recording cardiovascular activity. The changes in arterial pressure and heart rate were assessed during the following behaviors: grooming, feeding, changes in posture, orienting (elicited by an unexpected tapping sound on the front of the cat's cage), and rapid eye movement (REM) phase of sleep.

Histological Examination

The animals were killed by an intravenous injection of sodium pentobarbital. The brain was perfused with normal saline followed by 10% formalin. The brain was removed and placed in 10% formalin for at least 2 weeks. The localization of brain lesions was confirmed on frozen sections cut every 50 μ and stained for cells by the Nissl method. Obstruction of vessels within the lungs and kidneys by thrombi formed at the tips of the arterial and venous cannulas was assessed in three cats. No infarcts were observed.

RESULTS

ACUTE PHASE

Arterial Blood Pressure. Within 15 min after placement of bilateral electrolytic lesions in the NTS and termination of the anesthetic, the systolic, diastolic and the MAP began to rise in all cats. A record from an individual cat is shown in Fig. 1. Average response levels for the group are shown in Fig. 2 where the MAP rose to a maximum of 144 mm Hg at 1 hour which was significantly higher than that of the control group. Coincident with a decline of the average level of arterial pressure was the gradual appearance of intermittent, spontaneous fluctuations of the arterial pressure (Fig. 1D). These were seen by the 4th to 8th postoperative hour and gradually increased in frequency, duration and magnitude. By 24 hours lability of the arterial pressure was pronounced.

Heart Rate. The lesions also resulted in a significant elevation in heart rate (Figs. 1,2). The average heart rate increased more slowly than the MAP, reaching a maximum average level of 236 bpm at 7 hours after cessation of the anesthesia.

Venous Pressure. The venous pressure was unchanged following NTS lesions in all surviving cats (Figs. 1,2). In one cat, not included in the study, the venous pressure was substantially elevated. This cat died with pulmonary edema.

Respiration. Respiration ceased in two of the cats after placement of the lesions. These cats were mechanically ventilated 20-45 minutes before spontaneous respiration resumed. One of these cats and two others developed apneusis. After 1-2 hours, however, all cats were breathing normally.

CHRONIC PHASE

By 24 hours after the surgery, the cats with NTS lesions developed marked lability of the arterial pressure (Fig. 3). The lability was striking and included elevations, as well as falls, of arterial pressure that were sometimes as great as 100 mm Hg. In some instances the lability seemed to occur spontaneously in that it was not associated with any evident behavior nor to any identifiable stimulus. In other instances the lability was associated with occurrences of spontaneous or evoked behaviors.

The lability of the arterial pressure made it difficult to characterize the response levels over time by the usual method of selecting a few representative data points. We therefore analyzed the data by the generation of frequency histograms (8). This mode of analysis permitted us to examine the lability graphically and to determine numerically the MAP and standard deviation of each frequency histogram.

Since it appeared from inspection of the chart records that the lability of arterial pressure varied between daytime and nighttime, we analyzed separately the cardiovascular performance of cats at these two times of the day. The

lability appeared generally to be greatest during laboratory working hours (8 am - 8 pm) and least after closing the laboratory for the day (8 pm - 8 am). Therefore, the recordings were segregated into one of two general categories according to the hour of the day (8 am - 8 pm) or night (8 pm - 8 am) that the recordings were made. The exact hour of recording was selected to be as close as possible to the midpoint of the daytime or nighttime range (2 pm or 2 am).

Arterial Pressure - Daytime. During the daytime there were two changes in arterial pressure in cats with NTS lesions. First, the MAP increased, as indicated by the displacement of the frequency distributions of the cats with the lesions to the right of the distributions of the control cats (Fig. 4A,B,C). The MAP in the lesion group was 34 mm Hg higher than the MAP of the control group (Table 1A). Second, the lability of the arterial pressure increased markedly as seen by comparing the shapes of the frequency histograms of the cats with the lesions (Fig. 4C) to the distinctly different shapes of the frequency histograms of the control cats (Fig. 4B). In contrast to the prominent peak and narrow range (65-100 mm Hg) of the histograms of the control cats, the histograms of the cats with lesions are flattened and dispersed over 60-170 mm Hg. Numerically the increased lability is indicated by the size of the average standard deviation of the lesion group which was four times greater than the average standard deviation of the control group (Table 1B).

Heart Rate - Daytime. The heart rate was significantly increased during the day in cats with NTS lesions (Fig. 5A,B,C; Table 1C). While visual comparisons of the frequency histograms of the two groups suggest that the lability of the heart rate was reduced by NTS lesions, the standard deviations of in the lesion group did not differ significantly from the control group (Table 1D).

Arterial Pressure - Nighttime. The MAP at night in the cats with NTS lesions was significantly reduced from the daytime levels (Fig. 4D,E,F; Table 1A). However, it remained significantly elevated in comparison to the control group. The exaggerated lability of the MAP that was seen during the day was not present at night in cats with NTS lesions. Thus, the average standard deviation of these cats and the control cats did not differ significantly (Table 1B).

Heart Rate - Nighttime. In contrast to the MAP, the average heart rate of cats with NTS lesions seen during the day decreased only slightly at night so that it still remained significantly above the level of the control cats (Fig. 5A,B,C; Table 1C). The lability was unchanged from that of the control group (Table 1D).

EFFECT OF NTS LESIONS ON THE CARDIOVASCULAR RESPONSES DURING VARIOUS BEHAVIORS

Lesions of the NTS not only increased the range of spontaneous fluctuations of arterial pressure but also greatly exaggerated the normally small fluctuations asso-

ciated with various behaviors. Thus, the usually small elevation of arterial pressure associated with grooming or orienting were substantially enhanced (Fig. 6). Transient elevations of arterial pressure of up to 100 mm Hg were commonly seen.

NTS lesions also exaggerated naturally occurring reductions in arterial pressure. This was particularly noticeable during the REM phase of sleep. In the normal cat REM sleep is characterized by desynchronization of the EEG, rapid movement of the eyes, relaxation of the muscles of the neck and a moderate fall of arterial pressure (12). In cats with NTS lesions, hypotensive responses appeared which frequently reached a level of less than 50 mm Hg and which sometimes remained at that level for several minutes (Fig. 7). The electrophysiological events associated with REM sleep were unaffected by the NTS lesions.

EFFECTS OF NTS LESIONS ON BARORECEPTOR REFLEX ACTIVITY

In all cats, NTS lesions abolished the baroreceptor reflex as demonstrated by the absence of bradycardia after administration of a pressor dose of norepinephrine (Fig. 8).

LESION SITES

In all cats the electrodes were inserted in the NTS 0.5 mm rostral and caudal to the obex. The region of the NTS that was actually destroyed was located between about 1.0 mm rostral and 1.0 mm caudal to the obex. Representative lesions are shown in Fig. 9. In some cats the dorsal nucleus of the vagus, intercalary nucleus and the medial cuneate nucleus were minimally and variably damaged.

DISCUSSION

ACUTE PHASE

We have shown that bilateral electrolytic lesions of the NTS in cat, as in rat (9), result in the rapid development of arterial hypertension. The acute phase is characterized by stable elevations of arterial pressure of comparable magnitude in both species. However, the effects of NTS lesions on several other variables differed in the cat and rat. First, the cats exhibited a sustained tachycardia which the rats did not develop. Second, the cats generally did not develop elevated venous pressures or other evidence of cardiac failure. All cats, with the one exception previously noted, survived the acute phase after placement of the lesions. The rats, on the other hand, were in failure within 30 minutes after cessation of the anesthesia with elevated venous blood pressures, left ventricular and diastolic pressures, and reduced cardiac outputs. The rats uniformly succumbed to pulmonary edema after placement of the lesions within 4-6 hours.

The reasons why the cats survived the acute phase are uncertain. Conceivably, the elevation in heart rate was sufficient to maintain cardiac output, perhaps long enough for other and as yet unidentified compensatory mechanisms assist in gradually lowering the arterial pressure.

CHRONIC PHASE

Following the acute phase of stable hypertension, cats with bilateral NTS lesions entered the chronic phase consisting of: (a) marked minute to minute lability of arterial pressure; (b) a sustained elevation of MAP during the daytime; (c) exaggerated responsivity of the arterial pressure during various behaviors; and (d) sustained tachycardia.

The explanation accounting for the daily fluctuation in the amount of lability is uncertain but is probably related to interruption of the baroreceptor reflexes by placement of NTS lesions. Conceivably an endogenous, daily rhythm in the activity of sympathetic neurons is, under normal circumstances buffered by the baroreceptors, and interruption of the baroreceptor reflexes unmasks this rhythm. Alternatively, the fluctuations of arterial pressure could be secondary to changes in somatomotor activity and after interruption of the baroreceptor reflexes these fluctuations became greatly exaggerated.

Another explanation might be related to the fact that the cats with NTS lesions exhibited an enhanced cardiovascular reactivity to stimulation from their environment. Since in our study the cats were continuously exposed to the activities of the laboratory in which they were housed, the level of environmental stimulation was greater in the daytime when the laboratory was busy than at night, when it was quiet. In support of this explanation is our observation that the cardiovascular reactivity of the cats with lesions to inten-

tional environmental stimulation, for example during orienting, was much greater than that of the control cats.

The MAP of the group with NTS lesions was significantly elevated above the level of the control group during the day and the night while the lability of the pressure of the lesion group was significantly greater than the control group only during the day. These two facts suggest that the elevation of the MAP is not merely a statistical artifact caused when computing the mean by the addition of a few extremely high values of arterial pressure to otherwise normal or nearly normal values. Therefore, the sustained increase of the MAP produced by NTS lesions is, at least in part, independent of the increased lability and may reflect an enhanced tonic discharge of vasomotor neurons.

COMPARISON OF NTS LESIONS AND SINOARTIC DENERVATION

The changes in arterial pressure produced by NTS lesions are in all probability due to destruction of the arterial baroreceptor reflex mechanisms within the brain. First, the lesions always destroyed the middle third of the NTS, a major site of integration of arterial baroreceptor reflexes (1,4). Second, the reflex bradycardia elicited by elevation of the arterial pressure by norepinephrine was permanently abolished. Third, and indirectly, the disappearance of the major adjustments in cardiac rate in response to large fluctuation of arterial pressure during various behaviors in

cats with NTS lesions is entirely consistent with a loss of arterial baroreceptor responses. Since the NTS lesions abolished baroreceptor reflex responses and damaged the first synaptic relay within the brainstem of the fibers that project from the sinus and aortic baroreceptors (1,4), the results of NTS lesions and sinoaortic denervation should be compared. However, such comparisons with studies by others are difficult to make because of the differences in species examined, the environment in which the animals were tested and the methods of data analysis that were utilized. For example, in the only study of the effects of sinoaortic denervation in the unanesthetized cat, Guazzi and Zanchetti measured the MAP in cats that were isolated from acoustical and visual stimuli (12). They observed mean levels of arterial pressure that were much lower than the daytime levels measured by us, but comparable to our nighttime levels. The denervated cats also had exaggerated cardiovascular lability during REM sleep. Lability as a variable was not measured.

Lability appears to be a principle effect of sinoaortic denervation in the dog (6-8). In a recent study by Cowley, Liard and Guyton, analysis of frequency histograms of arterial pressure collected over 24 hour periods from dogs housed in a controlled environment, showed that sinoaortic denervation mainly increased the lability of the pressure and increased the MAP only slightly (8). Our results show a larger increase in the MAP and in the lability of the arterial pressure.

These comparisons from the literature indicate that sinoaortic denervation and NTS lesions have not only similarities but also differences. Whether such differences are real or an outcome of the variables of species, experimental conditions, and methods of data analysis cannot be established until NTS lesions and sinoaortic denervations are performed in the same species and under comparable conditions.

As a final note, it should be emphasized that there is an important difference between the procedures of sinoaortic denervation and placement of NTS lesions. While both procedures effectively block baroreceptor activity arising from the carotid sinus and aortic arch they differ in that the NTS lesions additionally destroy: (a) the terminus of baroreceptor afferent fibers that traverse the vagus nerve (1,4) and which are partially spared by the sinoaortic denervation (13,14), (b) the neurons upon which the baroreceptor afferent fibers terminate, and (c) projections from other brain areas onto neurons of the NTS (4). Thus, NTS lesions may be more effective than sinoaortic denervation in increasing the mean level and lability of the arterial pressure.

GENERAL IMPLICATIONS

The present study is the first example of the production of chronic, neurogenic hypertension in an animal model as a consequence of a localized brainstem lesion. The increased lability of the arterial pressure may reflect an imbalance,

caused by placement of the lesions, between excitatory and inhibitory systems which modulate sympathetic discharge. Normally, the reciprocal activity of these systems produces an orderly matching of cardiovascular events appropriate to the specific behaviors. After impairment of the inhibitory system by the lesions, an imbalance occurs that favors increased sympathetic discharge. Thus, the relatively small increases in sympathetic activity and arterial pressure normally seen in response to environmental stimulation and during various behaviors are unopposed by baroreceptor activity after NTS lesions and hence, the arterial pressure rises to abnormally high levels. When the environmental stimuli are reduced and the behavior ceases, the sympathetic discharge decreases and the arterial pressure returns to more nearly normotensive levels.

ACKNOWLEDGMENTS

We thank Lewis Tucker, Scott Bogatin and Nancy Shieh for their excellent technical assistance.

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LEGENDS

Fig. 1. Effect of NTS lesions during the acute phase on the arterial pressure, heart rate and central venous pressure of a single cat. Prelesion measurements (A) were taken just before the cat was anesthetized for the operation to place the lesions. The other panels show the cardiovascular responses at 15 minutes (B), 1 hour (C), 4 hours (D) and 8 hours (E) after placement of the lesions and termination of the anesthetic.

Fig. 2. Effect of NTS lesions during the acute phase on the mean arterial blood pressure (A), heart rate (B) and central venous pressure (C) of a group of cats. All cats were anesthetized for the period of time indicated by the shaded area. The data points on the left edge of the shaded area represent the responses recorded 1 hour before placement of lesions. The data points on the right edge of the shaded area represent the responses in the experimental group (filled circles, $n = 6$) after placement of NTS lesions or in the control group (open circles, $n = 7$) after sham lesions. At time 0, the anesthesia was stopped and the time course of cardiovascular activity for the next 8 hours was followed. Each data point signifies a mean value and the bars indicate \pm SE. The significance of differences from the control at each time point is represented by asterisks: $*=p<0.05$; $**=p<0.01$; $***=p<0.001$.

Fig. 3. Effect of NTS lesions during the chronic phase on the arterial pressure and heart rate of an individual cat. Prelesion tracing was taken 2 days before placement of the lesions when the cat was in quiet wakefulness and lying down. The postlesion result is taken 1 week after the lesion with the cat in the same behavioral state. Note the extreme lability of the arterial pressure.

Fig. 4. Frequency histograms of mean arterial pressure in normal cats and cats with NTS lesions during the daytime (lefthand panels) and the nighttime (righthand panels). Daytime frequency histograms: (A) Individual cat before and 1 week after placement of the lesions. (B) Overlay of 6 normal cats 1 week after the sham operation. (C) Overlay of 5 cats 1 week after placement of NTS lesions. Nighttime frequency histograms: (D) Individual cat before and 1 week after placement of the lesions. (E) Overlay of 5 normal cats 1 week after the sham operation. (F) Overlay of 4 cats 1 week after placement of NTS lesions.

Fig. 5. Frequency histograms of heart rate in normal cats and cats with NTS lesions during the daytime (lefthand panels) and the nighttime (righthand panels). Daytime frequency histograms: (A) Individual cat before and 1 week after placement of the lesions. (B) Overlay of 6 normal cats 1 week after the sham operation. (C) Overlay of 5 cats 1 week after placement of NTS lesions.

Nighttime frequency histograms: (D) Individual cat before and 1 week after placement of the lesions. (E) Overlay of 5 normal cats 1 week after the sham operation. (F) Overlay of 4 cats 1 week after placement of NTS lesions. The heart rates were recorded from the same cats as the mean arterial pressures shown in Fig. 4.

Fig. 6. Effect of NTS lesions on the changes in arterial pressure and heart rate associated with grooming (A) and orienting (B). The light vertical lines indicate the onset of the particular behavior. The responses were measured prior to placement of NTS lesions (control) and after placement of the lesions (NTS lesion) in the same cat.

Fig. 7. Facilitation by NTS lesions of the depressor response associated with rapid eye movement (REM) sleep in 2 cats. In cat #6, the cardiovascular and electrophysiological responses were recorded before and after placement of the lesions. In cat #12, the responses were recorded only after placement of the lesions. The cross-hatched bars at the bottom of the figure indicate the duration of the REM sleep episode.

Fig. 8. Comparison of the arterial pressure and heart rate responses to administration of one dose of norepinephrine (NE) prior to and after NTS lesions. Note the absence of bradycardia during the pressor response after placement of the lesions indicating abolition of baroreceptor reflexes.

Fig. 9. Location of lesions in the NTS that produced labile hypertension. The upper section (A) shows the extent of damage in the region of the NTS (arrows) at a level 0.5 mm rostral to the obex. The lower section (B) is from the same cat and shows the extent of damage (arrows) in the region of the NTS 0.5 mm caudal to the obex. Cresyl violet stain. Bar = 1 mm.

TABLE 1

Effects of lesions of the nucleus tractus solitarius (NTS) on the mean levels of the mean arterial pressure (A) and heart rate (C) and the standard deviations of the mean arterial pressure (B) and heart rate (D) during the day and night.

<u>A. MEAN ARTERIAL PRESSURE (mmHg)</u>				
	<u>Day</u>	<u>Night</u>	<u>% Difference (Night/Day)</u>	<u>P</u>
Control	*80±1.5 (6)	77±3.3 (5)	96	NS
Lesion	114±2.4 (5)	96±4.7 (4)	84	≤0.02
% Difference (Lesion/Control)	143	125	--	--
†P	≤0.001	≤0.02	--	--
<u>B. STANDARD DEVIATION OF ARTERIAL PRESSURE (mmHg)</u>				
	<u>Day</u>	<u>Night</u>	<u>% Difference (Night/Day)</u>	<u>P</u>
Control	4±0.4 (6)	5±0.4 (5)	125	NS
Lesion	18±2.3 (5)	6±0.5 (4)	33	≤0.05
% Difference (Lesion/Control)	450	120	--	--
P	≤0.001	NS	--	--
<u>C. HEART RATE (BPM)</u>				
	<u>Day</u>	<u>Night</u>	<u>%Difference (Night/Day)</u>	<u>P</u>
Control	154±8.1 (6)	149±6.4 (5)	97	NS
Lesion	191±5.6 (5)	181±1.4 (4)	95	NS
% Difference (Lesion/Control)	124	121	--	--
P	≤0.01	≤0.05	--	--
<u>D. STANDARD DEVIATION OF HEART RATE (BPM)</u>				
	<u>Day</u>	<u>Night</u>	<u>% Difference (Night/Day)</u>	<u>P</u>
Control	10±1.3 (6)	7±1.2 (5)	70	NS
Lesion	7±1.7 (5)	7±1.4 (4)	100	NS
% Difference (Lesion/Control)	70	100	--	--
P	NS	NS	--	--

All statistics were computed from the frequency histograms shown in Figs. 4 and 5.

*All Values Expressed as means ±SE; the number of cats in each group is enclosed in parentheses.

†P = Significance level. NS = not significant.

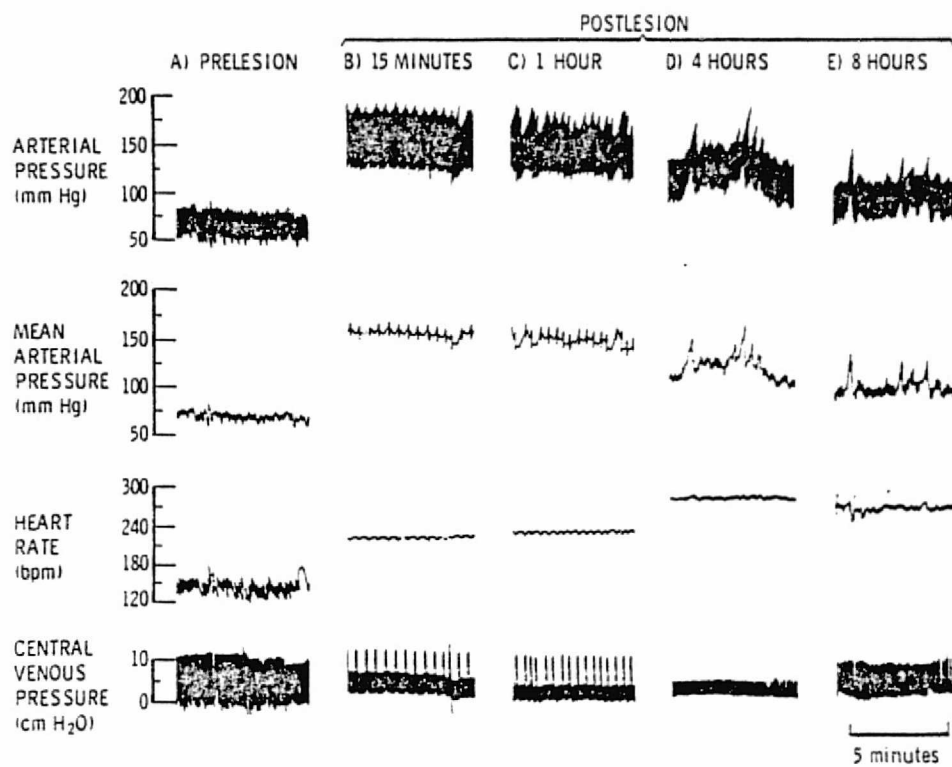


Figure 1.

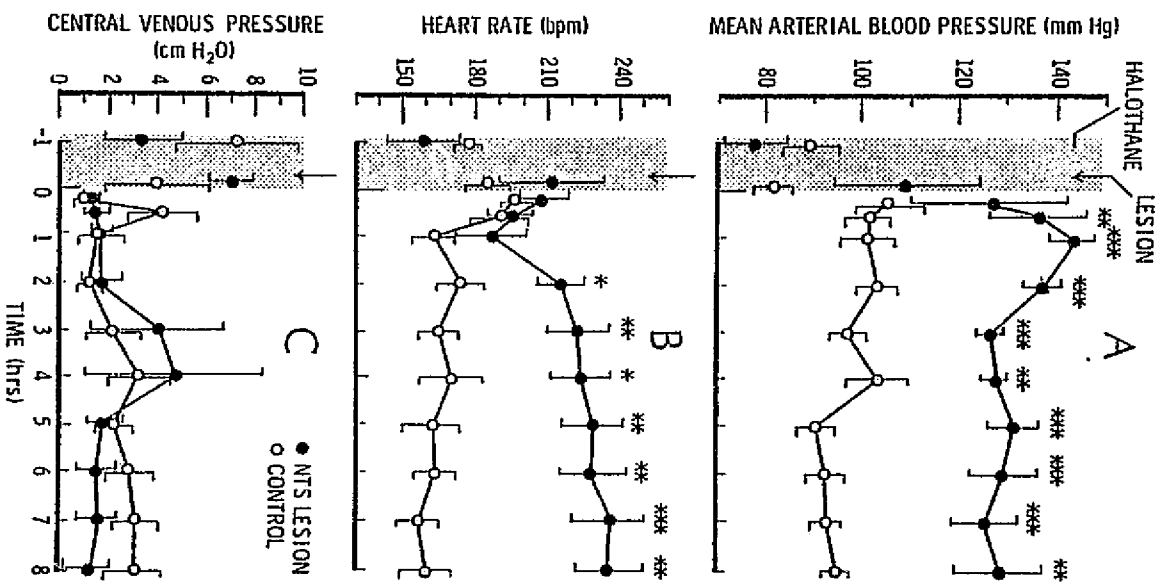


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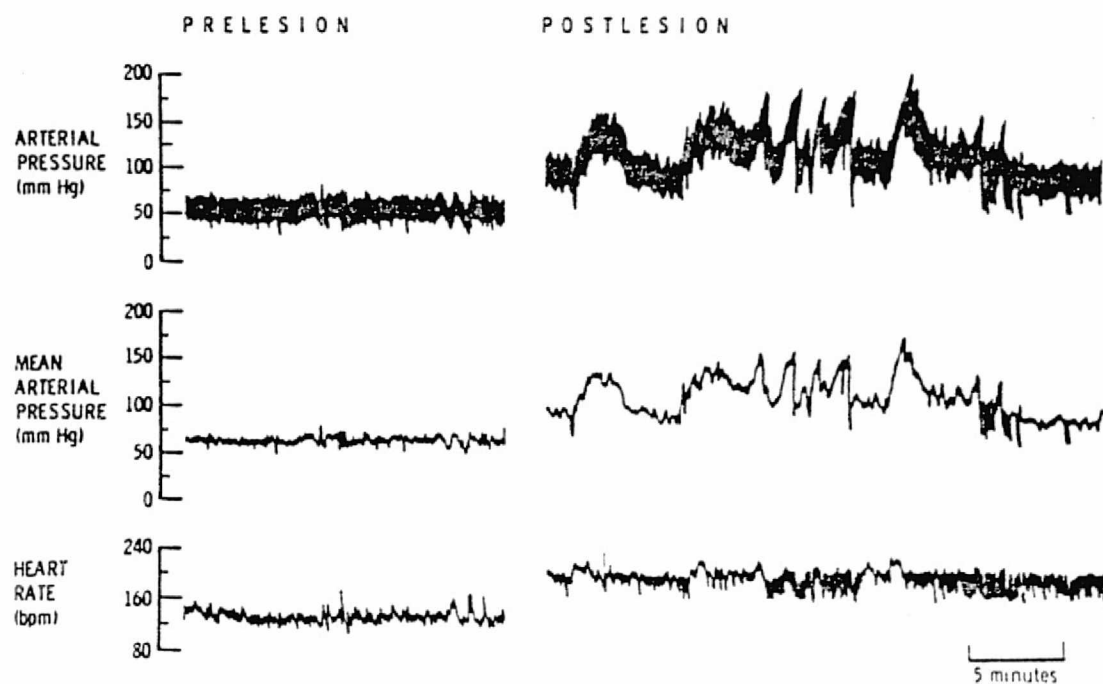


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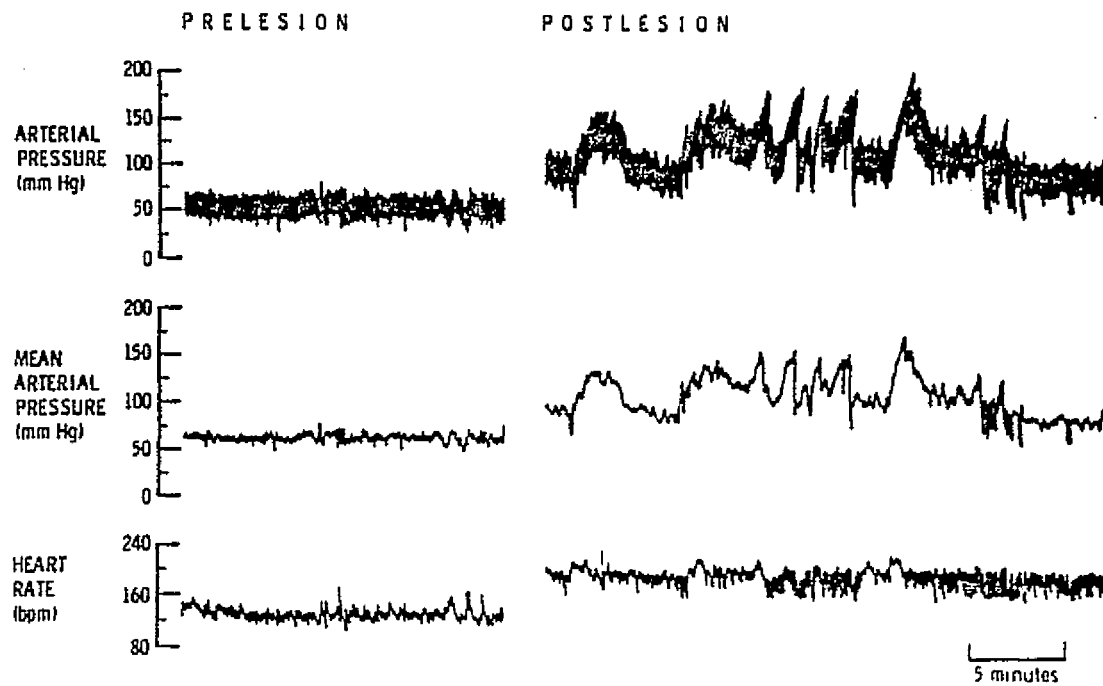


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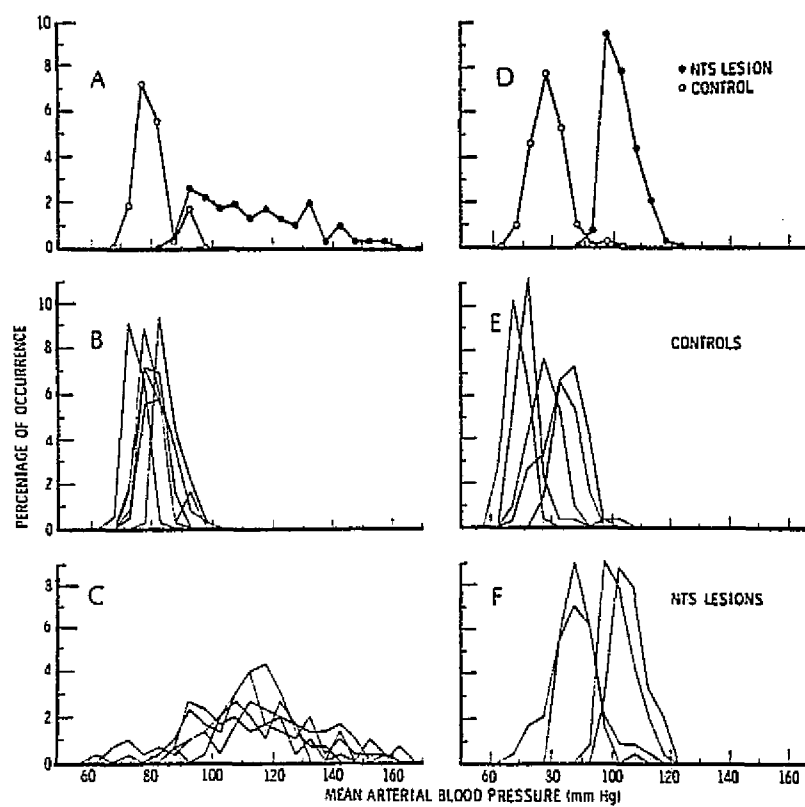


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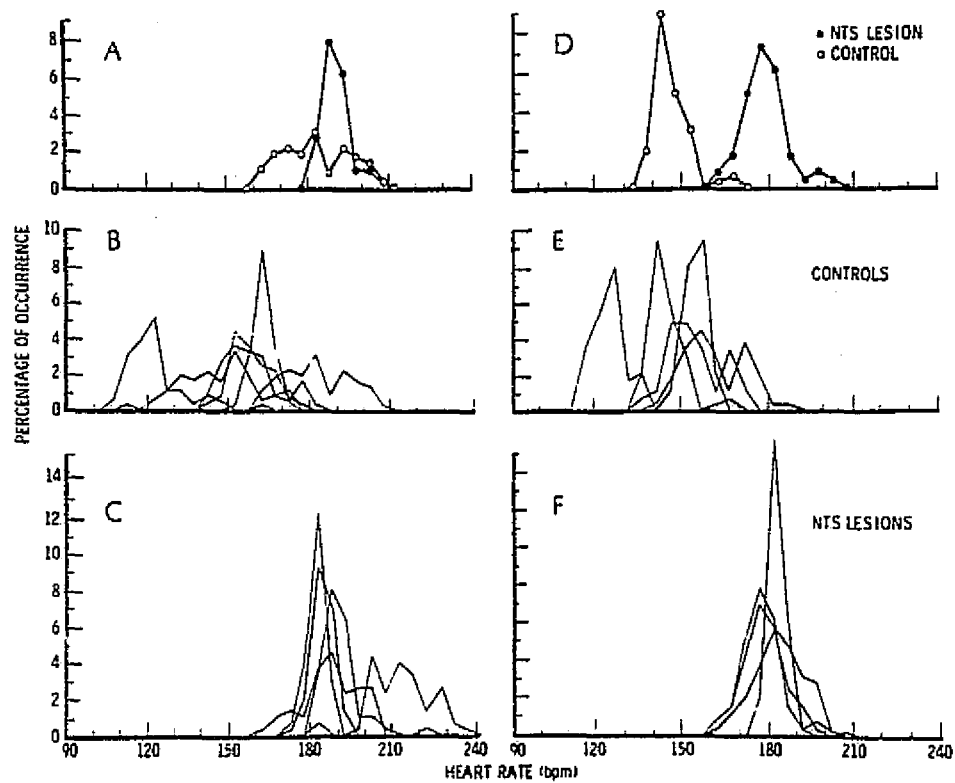


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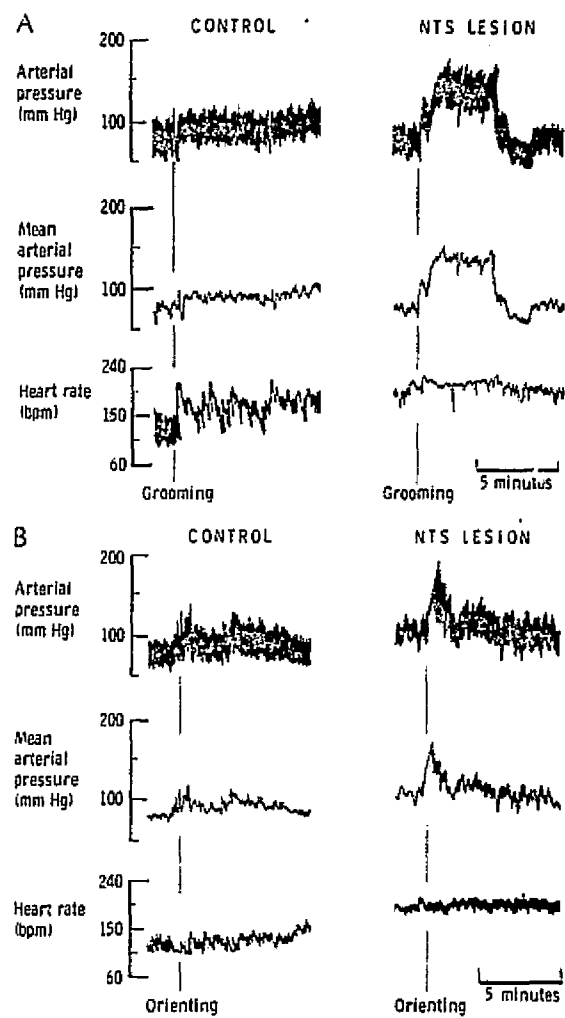


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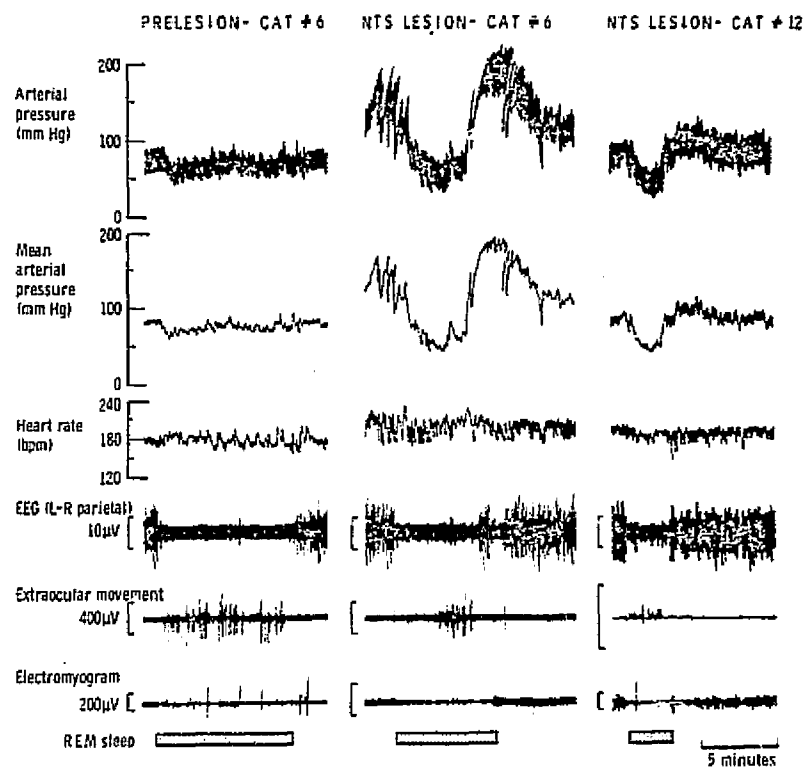


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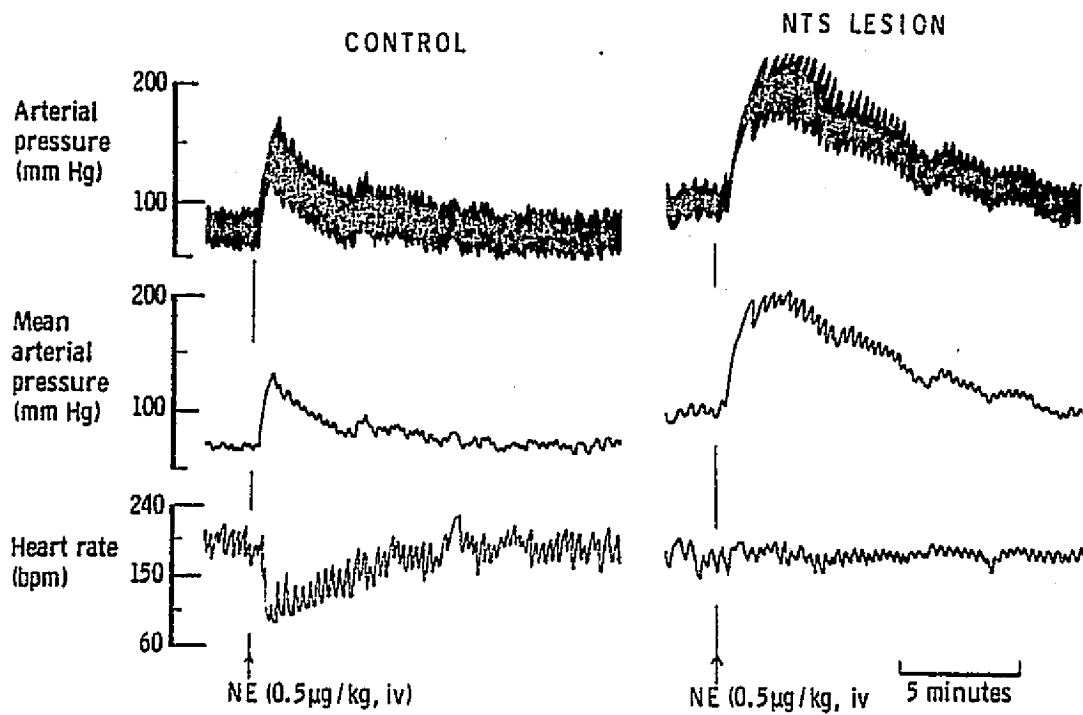


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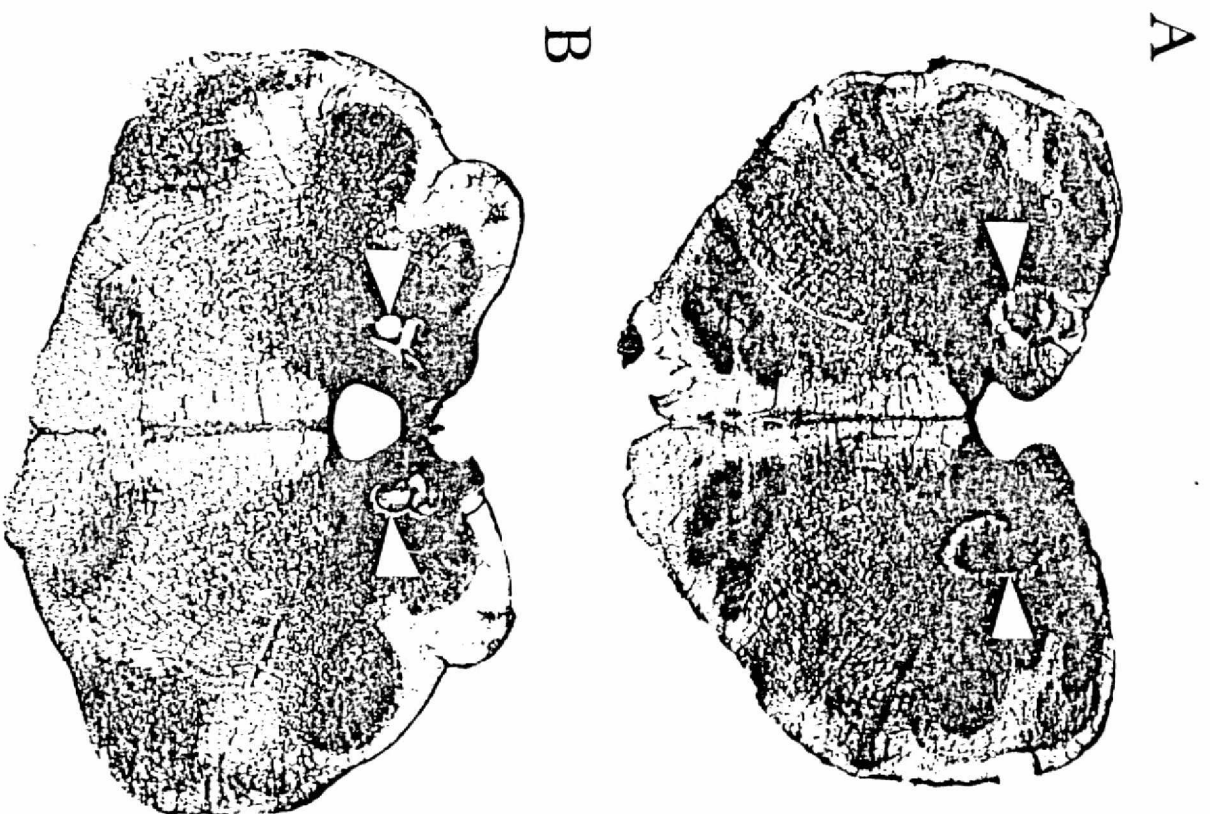


Figure 9.